**THE UNITED STATES PATENT AND TRADEMARK OFFICE**
**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

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| Appellant(s): | Perez-Camargo, et al. |
| Appl. No.: | 10/509,951 |
| Conf. No.: | 3093 |
| Filed: | October 4, 2004 |
| Title: | METHOD OF IMPROVING ABSORPTION OF VITAMIN E BY A PET ANIMAL |
| Art Unit: | 1612 |
| Examiner: | Snigdah Maewall |
| Docket No.: | 3714652-00509 |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**APPELLANTS' APPEAL BRIEF**

Sir:

Appellants submit this Appeal Brief in support of the Notice of Appeal filed on June 2, 2010. This Appeal is taken from the Final Rejection dated December 8, 2009 and the Advisory Action dated May 13, 2010.

## I. REAL PARTY IN INTEREST

The real party in interest for the above-identified patent application on Appeal is Nestec S.A. by virtue of an Assignment dated September 29, 2005 and recorded at reel 016613, frame 0473 in the United States Patent and Trademark Office.

## II. RELATED APPEALS AND INTERFERENCES

Appellants' legal representative and the Assignee of the above-identified patent application do not know of any prior or pending appeals, interferences or judicial proceedings which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision with respect to the above-identified Appeal.

## III. STATUS OF CLAIMS

Claims 35, 45, 48-52 and 57-64 are pending in the above-identified patent application. Claims 1-34, 36-44, 46-47, 53-56 and 65-68 were previously canceled without prejudice or disclaimer. Claims 35, 45, 48-52 and 57-64 stand rejected. Therefore, Claims 35, 45, 48-52 and 57-64 are being appealed in this Brief. A copy of the appealed claims is included in the Claims Appendix.

## IV. STATUS OF AMENDMENTS

A Non-Final Office Action was mailed on June 12, 2009, in which the Examiner rejected Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 under 35 U.S.C. §112, first and second paragraphs, and Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 under 35 U.S.C. §103. Appellants filed a Response to the Non-Final Office Action on August 19, 2009, in which Appellants amended Claims 35, 45, 52 and 61 and argued against the indefiniteness and obviousness rejections. A Final Office Action was mailed on December 8, 2009, in which the Examiner rejected Claim 35, 45, 48-52 and 57-64 under 35 U.S.C. §112, second paragraph, and Claims 35, 45, 48-52 and 57-64 under 35 U.S.C. §103. Appellants filed a Response to the Final Office Action on April 8, 2010, in which Appellants argued against the indefiniteness and obviousness rejections. An Advisory Action was mailed on May 13, 2010, in which the Examiner maintained the indefiniteness and obviousness rejections. Appellants filed a Notice of Appeal on June 2, 2010. Copies of the Non-Final Office Action, Final Office Action and Advisory Action are included in the Evidence Appendix as Exhibits A, B and C, respectively.

## V. SUMMARY OF CLAIMED SUBJECT MATTER

A summary of the invention by way of reference to the specification and/or figures for each of the independent claims is provided as follows:

Independent Claim 35 is directed to a method of improving or maintaining absorption of vitamin E in a cat that has, or is susceptible to, a vitamin E deficiency (page 3, lines 22-26; page 7, line 31), the method comprising the step of feeding the cat an effective amount of an edible composition to improve or maintain or promote the cat's lipid absorption capacity (page 7, lines 13-30), the edible composition comprising a pancreatic function-promoter comprising an acidifier (page 7, line 34-page 9, line 25), a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis (page 9, line 27-page 11, line 3), and an intestinal mucosa function-promoter comprising fish oil ranging between about 0.1% and 20% by weight of the edible composition on a dry matter basis (page 11, lines 5-32).

Independent Claim 52 is directed to a method of maintaining or improving the serum vitamin E level in a cat that has, or is susceptible to, a vitamin E deficiency (page 3, lines 22-26; page 7, line 31), comprising the step of feeding the cat an effective amount of an edible composition that maintains or improves the cat's lipid absorption capacity (page 7, lines 13-30), the edible composition comprising a pancreatic function-promoter (page 7, line 34-page 9, line 25), a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis (page 9, line 27-page 11, line 3), and an intestinal mucosa function-promoter comprising fish oil ranging between about 0.1% and 20% by weight of the edible composition on a dry matter basis (page 11, lines 5-32).

Independent Claim 61 is directed to a composition comprising a pancreatic function promoter comprising an acidifier (page 7, line 34-page 9, line 25), a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis (page 9, line 27-page 11, line 3), and an intestinal mucosa function promoter comprising fish oil ranging between about 0.1% and 20% by weight of the composition on a dry matter basis (page 11, lines 5-32).

Although specification citations are given in accordance with C.F.R. 1.192(c), these reference numerals and citations are merely examples of where support may be found in the

specification for the terms used in this section of the Brief. There is no intention to suggest in any way that the terms of the claims are limited to the examples in the specification. As demonstrated by the references numerals and citations below, the claims are fully supported by the specification as required by law. However, it is improper under the law to read limitations from the specification into the claims. Pointing out specification support for the claim terminology as is done here to comply with rule 1.192(c) does not in any way limit the scope of the claims to those examples from which they find support. Nor does this exercise provide a mechanism for circumventing the law precluding reading limitations into the claims from the specification. In short, the references numerals and specification citations are not to be construed as claim limitations or in any way used to limit the scope of the claims.

## VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Claims 35, 45, 48-52 and 57-64 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Appellants regard as the invention.

2. Claims 35, 45, 48-52 and 57-64 are rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,471,999 to Couzy et al. ("*Couzy*") in view of U.S. Patent No. 6,524,619 to Pearson et al. ("*Pearson*") and further in view of "Micronutrient status in patients with gastrointestinal disease" to Simpson et al. ("*Simpson*"), U.S. Patent No. 6,228,367 to Watson ("*Watson*"), U.S. Patent No. 6,160,007 to DeMichele et al. ("*DeMichele*"), and WO 01/62280 to Margolin et al. ("*Margolin*"). Copies of *Couzy*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* are included in the Evidence Appendix as Exhibits, D, E, F, G, H and I, respectively.

3. Claims 35, 45, 48-52 and 57-64 are rejected under 35 U.S.C. §103(a) as being unpatentable over WO 02/15719 to Fuchs et al. ("*Fuchs*") in view of *Pearson* and further in view of *Simpson*, *Watson*, *DeMichele* and *Margolin*. A copy of *Fuchs* is included in the Evidence Appendix as Exhibit J.

## VII. ARGUMENT

### A. LEGAL STANDARDS

#### 1. Definiteness under 35 U.S.C. §112, second paragraph

The standard for determining whether the definitiveness requirement is met under 35 U.S.C. § 112, ¶ 2 is "whether those skilled in the art would understand what is claimed when the claim is read in light of the Specification." *Orthokinetics Inc. v. Safety Travel Chairs Inc,* 1 U.S.P.Q. 2d 1081-1088 (Fed. Cir. 1986). "If the claims, read in light of the Specification, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the Courts can demand no more." *North American Vaccine Inc. v American Cyanamid Co.,* 28 U.S.P.Q. 2d 1333, 1339 (Fed. Cir. 1993). In this regard, "[p]atent law allows the inventor to be his own lexicographer ... [T]he specification aids in ascertaining the scope and meaning of the language employed in the claims inasmuch as words must be used in the same way in both the claims and the specification. *United States v. Teletronics, Inc.,* 8 U.S.P.Q. 2d 1217, 1220 (Fed. Cir. 1988). By statute, 35 U.S.C. 112, Congress has placed no limitations on how an applicant claims his invention, so long as the specification concludes with claims which particularly point out and distinctly claim that invention." *In re Pilkington,* 162 U.S.P.Q. 145, 148 (C.C.P.A. 1996).

#### 2. Obviousness under 35 U.S.C. §103

The Federal Circuit has held that the legal determination of an obviousness rejection under 35 U.S.C. § 103 is:

> whether the claimed invention as a whole would have been obvious to a person of ordinary skill in the art at the time the invention was made...The foundational facts for the prima facie case of obviousness are: (1) the scope and content of the prior art; (2) the difference between the prior art and the claimed invention; and (3) the level of ordinary skill in the art...Moreover, objective indicia such as commercial success and long felt need are relevant to the determination of obviousness...Thus, each obviousness determination rests on its own facts.

*In re Mayne,* 41 U.S.P.Q. 2d 1451, 1453 (Fed. Cir. 1997).

In making this determination, the Patent Office has the initial burden of proving a *prima facie* case of obviousness. *In re Rijckaert,* 9 F.3d 1531, 1532, 28 U.S.P.Q. 2d 1955, 1956 (Fed.

Cir. 1993). This burden may only be overcome "by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings." *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q. 2d 1596, 1598 (Fed. Cir. 1988). "If the examination at the initial stage does not produce a prima facie case of unpatentability, then without more the applicant is entitled to grant of the patent." *In re Oetiker*, 24 U.S.P.Q. 2d 1443, 1444 (Fed. Cir. 1992).

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the reference or references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *In re Fine*, 837 F.2d 1071, 5, U.S.P.Q.2d 1596 (Fed. Cir. 1988). Second, there must be a reasonable expectation of success. *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 U.S.P.Q. 375 (Fed. Cir. 1986). Finally, all of the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q., 580 (CCPA 1974).

Further, the Federal Circuit has held that it is "impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 23 U.S.P.Q.2d 1780, 1784 (Fed. Cir. 1992). "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." *In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988).

Moreover, the Federal Circuit has held that "obvious to try" is not the proper standard under 35 U.S.C. §103. *Ex parte Goldgaber*, 41 U.S.P.Q.2d 1172, 1177 (Fed. Cir. 1996). "An obvious-to-try situation exists when a general disclosure may pique the scientist curiosity, such that further investigation might be done as a result of the disclosure, but the disclosure itself does not contain a sufficient teaching of how to obtain the desired result, or that the claimed result would be obtained if certain directions were pursued." *In re Eli Lilly and Co.*, 14 U.S.P.Q.2d 1741, 1743 (Fed. Cir. 1990).

Of course, references must be considered as a whole and those portions teaching against or away from the claimed invention must be considered. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve Inc.*, 796 F.2d 443 (Fed. Cir. 1986). "A prior art reference may be considered to teach away when a person of ordinary skill, upon reading the reference would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the Applicant." *Monarch Knitting Machinery Corp. v. Fukuhara*

*Industrial Trading Co., Ltd.*, 139 F.3d 1009 (Fed. Cir. 1998), quoting, *In re Gurley*, 27 F.3d 551 (Fed. Cir. 1994).

B. THE CLAIMED INVENTION

Independent Claim 35 is directed methods of improving or maintaining absorption of vitamin E in a cat that has, or is susceptible to, a vitamin E deficiency. The methods include the step of feeding the cat an effective amount of an edible composition to improve or maintain or promote the cat's lipid absorption capacity. The edible composition has a pancreatic function-promoter comprising an acidifier, a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis, and an intestinal mucosa function-promoter including fish oil ranging between about 0.1% and 20% by weight of the edible composition on a dry matter basis.

Independent Claim 52 is directed to methods of maintaining or improving the serum vitamin E level in a cat that has, or is susceptible to, a vitamin E deficiency. The methods include the step of feeding the cat an effective amount of an edible composition that maintains or improves the cat's lipid absorption capacity. The edible composition has a pancreatic function-promoter, a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis, and an intestinal mucosa function-promoter including fish oil ranging between about 0.1% and 20% by weight of the edible composition on a dry matter basis.

Independent Claim 61 is directed to compositions having a pancreatic function promoter comprising an acidifier, a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis, and an intestinal mucosa function promoter comprising fish oil ranging between about 0.1% and 20% by weight of the composition on a dry matter basis.

C. CLAIMS 35, 37, 39-41, 43, 45, 48-52 AND 54-68 ARE SUFFICIENTLY DEFINITE TO SATISFY THE REQUIREMENTS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

The standard for determining whether the definitiveness requirement is met under 35 U.S.C. §112, second paragraph, is whether those skilled in the art would understand what is claimed when the claim is read in light of the specification. With respect to the presently claimed subject matter, Appellants respectfully disagree with the Examiner's assertion the terms "acidifier" and "fish oil" are unclear. See, Final Office Action, pages 2-3. Instead, Appellants respectfully submit that the skilled artisan would immediately understand the scope of the claims when read in view of the specification.

Appellants respectfully submit that the skilled artisan understands the term "acidifier" to be a compound that can lower the pH of another substance. The specification further discloses that examples of suitable acidifiers are citric acid and lactic acids. See, specification, page 8, lines 11-12. Further, Merriam Webster OnLine defines "acidifier" as one that acidifies; *especially* : a substance used to increase soil acidity. "Acidifies" is then defined as "1: to make acid; 2: to convert into an acid. See, Merriam Webster OnLine, definitions of "acidifier" and "acidifies." Since the skilled artisan would know that acidic substances generally have a pH of less than 7.0, the skilled artisan would appreciate that an "acidifier" would be a compound that can lower the pH of another substance to make the substance more acidic. Since basic compositions have higher pH levels, an "acidifier" would immediately be understood to be a compound that lowers the pH of a more basic substance to a more acidic substance.

In addition, the term "fish oil" is commonly known to be an oil derived from the tissues of oily fish. Further, Merriam Webster OnLine defines "fish oil" as "a fatty oil from the bodies of various fishes (as menhaden or sardines) that contains large amounts of unsaturated fatty acids and is used in making various products (as cosmetics and paints)." See, Merriam Webster OnLine, definition of "fish oil." As such, the skilled artisan would immediately appreciate what is meant by the term "fish oil."

As a result, the metes and bounds of the terms "acidifier" and "fish oil" are clear to the skilled artisan in view of the specification, the knowledge of the skilled artisan, as well as commonly used definitions of the terms. Based on at least these noted reasons, Appellants

believe that the pending claims fully comply with the requirements of 35 U.S.C. §112, second paragraph.

Accordingly, Appellants respectfully request that the rejection of Claims 35, 35, 48-52 and 57-64 under §112, second paragraph, be reconsidered and withdrawn.

D. THE FIRST REJECTION OF CLAIMS 35, 37, 39-41, 43, 45, 48-52 AND 54-68 UNDER 35 U.S.C. §103(a) SHOULD BE REVERSED

1. The Examiner has failed to establish a *prima facie* case of obviousness

Appellants respectfully submit that the obviousness rejection of Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 should be reversed because the Examiner has failed to establish a *prima facie* case of obviousness. In the Final Office Action, the Examiner asserts that the combination of *Couzy, Pearson, Simpson, Watson, DeMichele* and *Margolin* renders the claimed subject matter obvious. See, Final Office Action, pages 3-7. However, the Examiner has failed to establish a *prima facie* case of obviousness because the cited references fail to disclose each and every element of the present claims.

Independent Claims 35, 52 and 61 recite, in part, an edible composition comprising a pancreatic function-promoter comprising an acidifier, a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis, and an intestinal mucosa function-promoter comprising fish oil ranging between about 0.1% and 20% by weight of the edible composition on a dry matter basis. The edible composition can improve or maintain or promote a cat's lipid absorption capacity, which increases the absorption capacity of Vitamin E. In contrast, Appellants respectfully submit that even if combined the cited references fail to disclose or suggest each and every element of the present claims.

Vitamin E is a fat-soluble vitamin that is absorbed only with long chain fatty acids. A defect in either the absorption or digestion of lipid can therefore lead to deficiencies in this and other vitamins, due to their binding with unabsorbed fatty acids. See, Simpson, K W and Michel, K E. Micronutrient status in patients with gastrointestinal disease; Proceedings ACVIM,

Denver, Colo., pp. 651-653, 2001. Hence, a pet with low lipid digestibility is susceptible to several potential nutritional deficiencies, which can compromise its health.

Studies on senior cat nutrition have shown that a significant number of older pets--such as those above the age of 9 years-exhibit a decreased capacity to digest fat. Several scientific publications have likewise reported an age-related decrease in lipid digestibility in cats. See, Burkholder, W J., Age-related changes to nutritional requirements and digestive function in adult dogs and cats. JAVMA, Vol 215, No. 5, Sep. 1, 1999; Nicholson A, Watson A D J. Mercer J R., Fat malassimilation in three cats. Australian Veterinary Journal, Vol. 66, No. 4, April, 1989; Peachey S E, Dawson J M, Harper E J., The effects of aging on nutrient digestibility by cats fed beef tallow, sunflower oil or olive oil enriched diets. There can be any of a number of pathologies that can lead to poor digestibility of lipids. Malabsorption and maldigestion can occur from almost any diffuse disease of the intestine, from exocrine pancreatic insufficiency or from unknown causes. In the case of cats, pancreatitis occurs at a prevalence rate of about 0.15% to 3.5% and may account for some cases of poor fat digestibility. Diffuse intestinal diseases, such as intestinal lymphoma, small intestinal bacterial overgrowth, inflammatory bowel disease and liver disease, may also lead to reduced nutrient absorption in the small intestine.

In contrast to the present claims, Appellants respectfully submit that *Couzy*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* alone or in combination fail to disclose or suggest a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis as required independent Claims 35, 52 and 61. Applications also respectfully submit that *Couzy*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* fail to disclose or suggest the specific combination of the acidifier, taurine and fish oil in a single edible composition for improving or maintaining absorption of vitamin E in a cat as recited by independent Claims 35, 52 and 61.

Though *Couzy* mentions use of taurine, *Couzy* never teaches a level or range for taurine. *Pearson* is said to disclose that taurine can be used to enhance absorption of a drug. *Watson* and *DeMichele* fail to teach the use of, or even mention, any taurine. The Examiner relies on *DeMichele* for a disclosure of fish oil and *Simpson* and *Margolin* to arguably teach lipid assimilation. Nevertheless, these references fail to disclose or suggest the claimed range of the liver function-promoter and specific combination of components in accordance with Claims 35,

52 and 61. For at least the above-mentioned reasons, the cited references fail to disclose or suggest each and every element of the present claims.

Accordingly, Appellants respectfully request that the first obviousness rejection of Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 under 35 U.S.C. §103 be reconsidered and withdrawn.

2. Even if the Examiner has demonstrated a *prima facie* case of obviousness, Appellants have submitted a *Declaration* that rebuts any *prima facie* case of obviousness

"One way for a patent applicant to rebut a prima facie case of obviousness is to make a showing of 'unexpected results,' i.e., to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected." *In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995). Appellants have surprisingly found that an edible composition comprising an acidifier, taurine ranging between about 0.1% and about 1% by weight of the edible composition, and fish oil ranging between about 0.1% and 20% by weight of the edible composition creates a synergistic effect that improves the fat digestibility of the edible composition. As further shown by FIG. 1 of the specification, there is a direct correlation between fat digestibility and enhancement of the serum Vitamin E level. In other words, a composition that increases fat digestibility also increases the absorption capacity of Vitamin E by the body of the animal. See, specification, page 13, lines 20-25. This can reduce the effects of Vitamin E deficiency in a pet.

Appellants previously submitted a Declaration under 37 C.F.R. §1.132 ("*Declaration*" attached hereto as Exhibit K) that demonstrates the unexpected and synergistic results of administering an edible composition comprising an acidifier, taurine, and fish oil to a cat. As supported by the *Declaration*, a group of 20 cats with low fat digestibility (i.e., less than 80%) was fed diets to determine if there was an improvement in fat digestibility in old cats fed different diets containing combinations of pancreatic function promoters, liver function promoters, and intestinal mucosa function promoters, a "wet" diet (Diet A), a "dry" diet, (Diet B). It should be noted that the *Declaration* describes a more detailed version of the study of Example 1 in the specification.

The diets in the study contained a pancreatic function promoter (Diet A + citric acid), a liver function promoter (Diet A + taurine), an intestinal mucosa function promoter (Diet A + fish oil in the form of omega 3 oils), and a combination of the promoters (Diet C) were formulated and fed to cats using the procedure similar to that given in Example 1 of the above-identified patent application. The citric acid in the diets was in an amount of approximately 0.1% by weight. The taurine in the diets was in an amount of approximately 0.8% by weight. The fish oil in the diets was in an amount of approximately 3% by weight.

As supported by the *Declaration*, the control diets (Diet A and Diet B) showed a fat digestibility of about 61% and 63%, respectively, as shown in Figure 1 of the *Declaration*. There was no significant difference between fat digestibility of a wet diet and a dry diet. This confirms that the digestibility of wet and dry diets is substantially the same and that diet is not a factor in evaluating digestibility. Diet A + citric acid, Diet A + taurine, and Diet A + fish oil showed an increase in fat digestibility of 6.6%, 6.1% and 5.5%, respectively, when compared to the control diets. However, surprisingly, the combination of the three promoters showed a much more pronounced and synergistic effect on fat digestibility. The combination (Diet C) showed an increase in fat digestibility of 17.5%.

As supported by the *Declaration*, in old cats with reduced fat digestibility (<80%), the presence of a single pancreatic function promoter (acidifier), a single liver function promoter (taurine), or a single intestinal mucosa function promoter (omega 3 oils) improved the level of fat digestibility (around 5.5 to 6.6%). However, none of these diets increased the level of fat digestibility above 80%, the level considered as normal. When the inventors provided the same old cats with a diet that contains a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils), the improvement in the level of fat digestibility is more dramatic (around 17.5%). Only with this diet did the old cats reach a level of fat digestibility that was considered normal (above 80%). This is a dramatic effect; not even in young healthy cats can fat digestibility be 100%. Moreover, no digestive system is 100% efficient (every meal produces some fecal content).

As supported by the *Declaration*, the results are surprising and unexpected when the percentage of cats that showed an increase in fat digestibility is analyzed as shown in Figure 2 of the *Declaration*. The percent of cats that had an improved fat digestibility when administered the promoters in combination was 90%, as compared to the 67% to 75% for the promoters alone.

About 20% more cats will have increased fat digestibility if administered a combination or promoters than if administered one of the promoters alone. Thus, one critical discovery is that the number of cats that benefit from a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils) is much greater than the number of cats that benefit from a single promoter. Figure 2 shows that 90% of the cats improved their fat digestibility, versus only 75% when fed a diet with a single pancreatic function promoter (acidifier), 67 % with a single liver function promoter (Taurine), or 67% with a single intestinal mucosa function promoter (omega 3 oils).

As supported by the *Declaration*, the decrease in fat digestibility in old cats is a complex problem that involves a decrease in pancreatic function, liver function, and/or intestinal mucosal function. In most cases, as is frequent with old age, there is not a clear and consistent malfunction, but a concomitant and interrupted decrease of multiple organ efficiency or malfunction. The inventors made a critical discovery in that the number of cats that benefit from an edible composition including a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils) is much greater than the number of cats that benefit from a single promoter. The beneficial effects of the edible composition lead to an increase in fat digestibility in the cat that also correlates to an increase in the absorption capacity of Vitamin E by the cat.

The *Declaration* further demonstrates that *Couzy*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* alone or in combination fail to disclose or suggest a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis as required independent Claims 35, 52 and 61, and as discussed above. Applications also respectfully submit that *Couzy*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* fail to disclose or suggest the specific combination of the acidifier, taurine and fish oil in a single edible composition for improving or maintaining absorption of vitamin E in a cat as recited by independent Claims 35, 52 and 61.

In the Advisory Action, the Examiner asserts that "no comparative data for individual cat[s] is shown in terms of lipid absorption." See, Advisory Action, page 2. However, Appellants note that over a period of several years, the inventors studied the digestibility of numerous diets in a significant number of cats ($n > 1000$). The inventors did batteries of digestibility studies on a continuous basis following Association of American Feed Control

Officials ("AAFCO") protocols. These tests showed that one third of cats over the age of 12 years suffer from low fat digestibility. See, *Declaration*, pages 2-3. As disclosed in Example 1, 20 cats with low fat digestibility underwent different digestibility tests using different diets, as described in detail in the specification and the *Declaration*. Appellants submit that is it not necessary to provide "comparative data for individual cat[s] . . . in terms of lipid absorption." Instead, Appellants have organized the experimental data in an easily readable format to clearly illustrate the unexpected and synergistic results that are obtained when cats are fed the presently claimed compositions. Indeed, the simple fact that Appellants have not set forth data for each individual cat does not cause the Examples in the specification or the studies summarized in the *Declaration* to be "insufficient evidence," as is alleged by the Examiner.

Moreover, Appellants also note that patentability does not depend on demonstration of explicit data results. Indeed, an example may be "working" or "prophetic." A working example is based on work actually performed. A prophetic example describes an embodiment of the invention based on predicted results rather than work actually conducted or results actually achieved. An applicant need not have actually reduced the invention to practice prior to filing. See, MPEP 2164.02. Therefore, Appellants respectfully submit that, even if data is not present for each individual cat, such data is not required. Instead, Appellants have clearly illustrated the unexpected and synergistic results that are obtained when cats are fed the presently claimed compositions.

The Examiner further states that "it is not clear which specific group of fat or lipid absorption is the applicant referring to especially in the absence of statistical data to show fat absorption." See, Advisory Action, page 3. In response, Appellants submit that it is not necessary for Appellants to test each and every specific group of fats or lipids to demonstrate the unexpected and synergistic results that are obtained when cats are fed the presently claimed composition. Instead, Appellants submit that the Examples in the specification and the attached *Declaration* clearly demonstrate that cats are able to maintain their weight better (e.g., increase fat digestibility) when fed diets corresponding to the present claims when compared to control diets. To require Appellants to demonstrate results for each and every fat/lipid would be unduly burdensome for Appellants. In contrast, Appellants submit that the data presented in the specification and *Declaration* are more than sufficient to demonstrate the unexpected and synergistic results that are obtained when cats are fed the compositions of the present claims.

In sum, *Couzy*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* alone or in combination fail to disclose or suggest each and every element of independent Claims 35, 52 and 61. Moreover, the cited references fails to even recognize the advantages, unexpected benefits and/or properties of the edible composition and methods of feeding the composition to a cat in accordance with the present claims. Consequently, independent Claims 35, 52 and 61, along with the claims that depend from Claims 35, 52 and 61, are novel and non-obvious over the cited references.

For at least the above-mentioned reasons, Appellants respectfully submit that the cited references fail to disclose or suggest each and every element of the present claims.

Accordingly, Appellants respectfully request that the first rejection of Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 under 35 U.S.C. §103 should be reconsidered and withdrawn.

## E. THE SECOND REJECTION OF CLAIMS 35, 37, 39-41, 43, 45, 48-52 AND 54-68 UNDER 35 U.S.C. §103(a) SHOULD BE REVERSED

### 1. The Examiner has failed to establish a *prima facie* case of obviousness

Appellants respectfully submit that the second obviousness rejection of Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 should be reversed because the Examiner has failed to establish a *prima facie* case of obviousness. In the Final Office Action, the Examiner asserts that the combination of *Fuchs*, *Simpson*, *Watson*, *DeMichele*, and *Margolin* renders the claimed subject matter obvious. See, Final Office Action, pages 8-11. However, the Examiner has failed to establish a *prima facie* case of obviousness because the cited references fail to disclose each and every element of the present claims.

As discussed above, independent Claims 35, 52 and 61 recite, in part, an edible composition comprising a pancreatic function-promoter comprising an acidifier, a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis, and an intestinal mucosa function-promoter comprising fish oil ranging between about 0.1% and 20% by weight of the edible composition on a dry matter basis. The edible composition can improve or maintain or promote a cat's lipid absorption capacity, which increases the absorption capacity of Vitamin E. In contrast, Appellants

respectfully submit that even if combined the cited references fail to disclose or suggest each and every element of the present claims.

In contrast to the present claims, Appellants respectfully submit that *Fuchs*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* alone or in combination fail to disclose or suggest a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis as required independent Claims 35, 52 and 61. Applications also respectfully submit that *Fuchs*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* fail to disclose or suggest the specific combination of the acidifier, taurine and fish oil in a single edible composition for improving or maintaining absorption of vitamin E in a cat as recited by independent Claims 35, 52 and 61.

*Fuchs* teaches use of emulsifiers and taurine, but without any usage levels. *Pearson* is said to disclose that taurine can be used to enhance absorption of a drug. *Watson* and *DeMichele* fail to teach the use of or even mention any taurine. The Examiner relies on *DeMichele* for a disclosure of fish oil and *Simpson* and *Margolin* to arguably teach lipid assimilation. Nevertheless, these references fail to disclose or suggest the claimed range of the liver function-promoter and specific combination of components in accordance with Claims 35, 52 and 61.

For at least the above-mentioned reasons, the cited references fail to disclose or suggest each and every element of the present claims.

Accordingly, Appellants respectfully request that the second obviousness rejection of Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 under 35 U.S.C. §103 be reconsidered and withdrawn.

2. Even if the Examiner has demonstrated a *prima facie* case of obviousness, Appellants have submitted a *Declaration* that rebuts any *prima facie* case of obviousness

As is discussed above, Appellants previously submitted a *Declaration* that demonstrates the unexpected results of administering an edible composition comprising an acidifier, taurine, and fish oil to a cat. As supported by the *Declaration*, a group of 20 cats with low fat digestibility (i.e., less than 80%) was fed diets to determine if there was an improvement in fat digestibility in old cats fed different diets containing combinations of pancreatic function promoters, liver function promoters, and intestinal mucosa function promoters, a "wet" diet (Diet

A), a "dry" diet, (Diet B). It should be noted that the *Declaration* describes a more detailed version of the study of Example 1 in the specification.

The diets in the study contained a pancreatic function promoter (Diet A + citric acid), a liver function promoter (Diet A + taurine), an intestinal mucosa function promoter (Diet A + fish oil in the form of omega 3 oils), and a combination of the promoters (Diet C) were formulated and fed to cats using the procedure similar to that given in Example 1 of the above-identified patent application. The citric acid in the diets was in an amount of approximately 0.1% by weight. The taurine in the diets was in an amount of approximately 0.8% by weight. The fish oil in the diets was in an amount of approximately 3% by weight.

As supported by the *Declaration*, the control diets (Diet A and Diet B) showed a fat digestibility of about 61% and 63%, respectively, as shown in Figure 1 of the *Declaration*. There was no significant difference between fat digestibility of a wet diet and a dry diet. This confirms that the digestibility of wet and dry diets is substantially the same and that diet is not a factor in evaluating digestibility. Diet A + citric acid, Diet A + taurine, and Diet A + fish oil showed an increase in fat digestibility of 6.6%, 6.1% and 5.5% respectively when compared to the control diets. However, surprisingly, the combination of the three promoters showed a much more pronounced affect on fat digestibility. The combination (Diet C) showed an increase in fat digestibility of 17.5%.

As supported by the *Declaration*, in old cats with reduced fat digestibility (<80%), the presence of a single pancreatic function promoter (acidifier), a single liver function promoter (taurine), or a single intestinal mucosa function promoter (omega 3 oils) improved the level of fat digestibility (around 5.5 to 6.6%). However, none of these diets increased the level of fat digestibility above 80%, the level considered as normal. When the inventors provided the same old cats with a diet that contains a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils), the improvement in the level of fat digestibility is more dramatic (around 17.5%). Only with this diet did the old cats reach a level of fat digestibility that was considered normal (above 80%). This is a dramatic effect; not even in young healthy cats can fat digestibility be 100%. Moreover, no digestive system is 100% efficient (every meal produces some fecal content).

As supported by the *Declaration*, the results are surprising and unexpected when the percentage of cats that showed an increase in fat digestibility is analyzed as shown in Figure 2 of

the *Declaration*. The percent of cats that had an improved fat digestibility when administered the promoters in combination was 90%, as compared to the 67% to 75% for the promoters alone. About 20% more cats will have increased fat digestibility if administered a combination or promoters than if administered one of the promoters alone. Thus, one critical discovery is that the number of cats that benefit from a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils) is much greater than the number of cats that benefit from a single promoter. Figure 2 shows that 90% of the cats improved their fat digestibility, versus only 75% when fed a diet with a single pancreatic function promoter (acidifier), 67 % with a single liver function promoter (Taurine), or 67% with a single intestinal mucosa function promoter (omega 3 oils).

As supported by the *Declaration*, the decrease in fat digestibility in old cats is a complex problem that involves a decrease in pancreatic function, liver function, and/or intestinal mucosal function. In most cases, as is frequent with old age, there is not a clear and consistent malfunction, but a concomitant and interrupted decrease of multiple organ efficiency or malfunction. The inventors made a critical discovery in that the number of cats that benefit from an edible composition including a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils) is much greater than the number of cats that benefit from a single promoter. The beneficial effects of the edible composition lead to an increase in fat digestibility in the cat that also correlates to an increase in the absorption capacity of Vitamin E by the cat.

The *Declaration* further demonstrates that *Fuchs*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* alone or in combination fail to disclose or suggest a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis as required independent Claims 35, 52 and 61. Applications also respectfully submit that *Fuchs*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* fail to disclose or suggest the specific combination of the acidifier, taurine and fish oil in a single edible composition for improving or maintaining absorption of vitamin E in a cat as recited by independent Claims 35, 52 and 61.

*Fuchs* teaches use of emulsifiers and taurine, but without any usage levels. *Pearson* is said to disclose that taurine can be used to enhance absorption of a drug. *Watson* and *DeMichele* fail to teach the use of or even mention any taurine. The Examiner relies on *DeMichele* for a

disclosure of fish oil and *Simpson* and *Margolin* to arguably teach lipid assimilation. Nevertheless, these references fail to disclose or suggest the claimed range of the liver function-promoter and specific combination of components in accordance with Claims 35, 52 and 61.

In sum, *Fuchs*, *Pearson*, *Simpson*, *Watson*, *DeMichele* and *Margolin* alone or in combination fail to disclose or suggest each and every element of independent Claims 35, 52 and 61. Moreover, the cited references fails to even recognize the advantages, unexpected benefits and/or properties of the edible composition and methods of feeding the composition to a cat in accordance with the present claims. Consequently, independent Claims 35, 52 and 61, along with the claims that depend from Claims 35, 52 and 61, are novel and non-obvious in view of the cited references.

For at least the above-mentioned reasons, Appellants respectfully submit that the cited references fail to disclose or suggest each and every element of the present claims.

Accordingly, Appellants respectfully request that the second rejection of Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 under 35 U.S.C. §103 should be reconsidered and withdrawn.

## VIII. CONCLUSION

Appellants respectfully submit that Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 meet the requirements of 35 U.S.C. §112, second paragraph. Appellants further submit that the Examiner has failed to establish a *prima facie* case of obviousness under 35 U.S.C. §103 and that, even if the Examiner has established a *prima facie* case of obviousness, Appellants have rebutted any showing of obviousness by demonstrating unexpected results. Accordingly, Appellants respectfully submit that the indefiniteness and obviousness rejections are erroneous in law and in fact and should, therefore, be reversed by this Board.

The Director is authorized to charge $540 for the Appeal Brief and any additional fees which may be required, or to credit any overpayment to Deposit Account No. 02-1818. If such a withdrawal is made, please indicate the Attorney Docket No. 3714652-00509 on the account statement.

Respectfully submitted,

K&L GATES LLP

BY _____

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Dated: July 28, 2010

## CLAIMS APPENDIX

## PENDING CLAIMS ON APPEAL OF U.S. PATENT APPLICATION SERIAL NO. 10/509,951

35. A method of improving or maintaining absorption of vitamin E in a cat that has, or is susceptible to, a vitamin E deficiency, the method comprising the step of feeding the cat an effective amount of an edible composition to improve or maintain or promote the cat's lipid absorption capacity, the edible composition comprising a pancreatic function-promoter comprising an acidifier, a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis, and an intestinal mucosa function-promoter comprising fish oil ranging between about 0.1% and 20% by weight of the edible composition on a dry matter basis.

45. A method according to claim 35 wherein the component has a fatty acid profile selected to improve intestinal absorption.

48. A method according to claim 35 wherein the composition is administered as a nutritionally balanced, ready-to-eat meal.

49. A method according to claim 48 wherein the meal comprises a dried pet food kibble.

50. A method according to claim 35 wherein the composition is administered as a meal supplement.

51. A method according to claim 50 wherein the meal supplement is in the form of a treat.

52. A method of maintaining or improving the serum vitamin E level in a cat that has, or is susceptible to, a vitamin E deficiency, comprising the step of feeding the cat an effective amount of an edible composition that maintains or improves the cat's lipid absorption capacity, the edible composition comprising a pancreatic function-promoter, a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis, and an intestinal mucosa function-promoter comprising fish oil ranging between about 0.1% and 20% by weight of the edible composition on a dry matter basis.

57. A method according to claim 52 wherein the component has a fatty acid profile selected to improve intestinal absorption.

58. A method according to claim 52 wherein the composition is administered as a nutritionally balanced, ready-to-eat meal.

59. A method according to claim 58 wherein the meal comprises a dried pet food kibble.

60. A method according to claim 58 wherein the meal is administered daily.

61. A composition comprising a pancreatic function promoter comprising an acidifier, a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis, and an intestinal mucosa function promoter comprising fish oil ranging between about 0.1% and 20% by weight of the composition on a dry matter basis.

62. The composition of claim 61 wherein the composition is a nutritionally balanced, ready-to-eat pet meal.

63. The composition of claim 62 wherein the meal is a wet pet food.

64. The composition of claim 62 wherein the meal is a dry pet food.

## EVIDENCE APPENDIX

EXHIBIT A: Non-Final Office Action dated June 12, 2009

EXHIBIT B: Final Office Action dated December 8, 2009

EXHIBIT C: Advisory Action dated May 13, 2010

EXHIBIT D: U.S. Patent No. 6,471,999 to Couzy et al. ("*Couzy*")

EXHIBIT E: U.S. Patent No. 6,524,619 to Pearson et al. ("*Pearson*")

EXHIBIT F: "Micronutrient status in patients with gastrointestinal disease" to Simpson et al. ("*Simpson*")

EXHIBIT G: U.S. Patent No. 6,228,367 to Watson ("*Watson*")

EXHIBIT H: U.S. Patent No. 6,160,007 to DeMichele et al. ("*DeMichele*")

EXHIBIT I: WO 01/62280 to Margolin et al. ("*Margolin*")

EXHIBIT J: WO 02/15719 to Fuchs et al. ("*Fuchs*")

EXHIBIT K: *Declaration* of Gerardo Perez-Camargo under 37 C.F.R. §1.132

## RELATED PROCEEDINGS APPENDIX

None.

# EXHIBIT A

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 10/509,951 | 10/04/2004 | Gerardo Perez-Camargo | 115808-509 | 3093 |

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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

chicago.patents@klgates.com

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/509,951 | PEREZ-CAMRGO,GERARDO | |
| | Examiner | Art Unit | |
| | Snigdha Maewall | 1612 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *05 March 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *35,37,39-41,43,45,48-52 and 54-68* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *35, 37, 39-41, 43, 45, 48-52 and 54-68* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

### *Summary*

1. Receipt of Applicant's arguments/remarks and amended claims all filed on 03/05/09 is acknowledged.

Claims 1-34, 36, 38, 42, 44, 46-47 and 53 have been cancelled.

Claims **35, 37, 39-41, 43, 45, 48-52 and 54-68** are pending in this application and claims **35, 37, 39-41, 43, 45, 48-52 and 54-68** will be prosecuted on the merits..

### *Claim Rejections - 35 USC § 112*

2. The following is a quotation of the first paragraph of 35 U.S.C. 112:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 1 recites the limitation that a liver function promoter comprises between about 0.1% to about 1.00% by weight of the edible composition. The specification on page 9, lines 30-35 discloses weight of only taurine. The amount is not disclosed for any

kind of liver function promoter as claimed such as glutathione promoters, glutathione or emulsifiers. This is a new matter rejection.

The limitation that a pet animal that has or is susceptible to, a vitamin deficiency is not disclosed in the specification. The specification only discloses a pet which is cat, see page 18. this is a new matter rejection.

***Claim Rejections - 35 USC § 112***

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5 Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 43 and 45 recite the limitation anti-inflammatory agent, claim 43 recites the limitation omega-3 fatty acid and a fatty acid having a profile specially selected to improve absorption makes the claim indefinite, no metes and bounds of claim can be deduced as recited. Appropriate corrections are requested. Claim 43 recites the limitation anti-inflammatory agent twice which is not further limiting in a Markush group. Applicants have not described in claim 1 any specific pancreatic function promoter or intestinal mucosa function promoter, it is not clear how an intestinal mucosa function promoter or pancreatic function promoter will increase lipid absorption and further improve vitamin E absorption. Claim 39 recites the limitation "emulsifiers, vitamins,

minerals and glutathione promoters. The metes and bounds of claim are not defined. Claim 41 recites the limitation "agent and carrier"., Appropriate correction is required.

***Claim Rejections - 35 USC § 103***

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 are rejected 35 U.S.C. 103(a) as being unpatentable over US Patent No. 6,471,999 in view of US 5,290,571 ('571) or US 5,451,412 ('412) and further in view of (Simpson, KW and Michel, KE. Micronutrient status in patients with gastrointestinal disease. Proceedings ACVIM, Denver, CO, pp. 651-653, 2001), (Suzuki et al. Gastroenterology 1999; 116:431-437 7), (W0 01/62280) and (USP 6,228,367).

'999 teach a pet milk powder as nutritional milk those results in reduced gastrointestinal intolerance (abstract). '999 teaches that the milk powder when administered in an effective amount with the nutritional composition reduces gastrointestinal intolerance and that it may further comprise one or more lipid source, protein source, vitamins and minerals, and teaches a specific aspect which comprises lactose (of micro-organism origin), lactase, taurine, arginine and choline (claims 1-9; col. 2, lines 9-lines 26).

'999 teach including an alkali in the milk-based powder, (an alkali as pancreatic function promoter as claimed) which slows the pH, drop in the gastrointestinal tract (col. 2, lines 53-55). '999 teaches that a protein source of whey protein and further supplemented with taurine and a probiotic micro-organism which beneficially effects the host by improving its intestinal microbial balance, such as lactic acid (col. 3, lines 25-40). '999 teaches chicory fibers, inulin, fructooligosaccharides with the probiotic micro-organism (intestinal function promoter as claimed) have a symbiotic relationship for promoting beneficial effects (col. 4, lines 9-14).

'999 teaches that the amount of nutritional composition is to be fed to a mammal each day depends on factors such as age, type of mammal (dogs and cats), and other nutritional sources (col. 4, lines 25-36). Examples 1 and 2 teach mixing the milk powder, galactosidase (lactase amino), **vitamins** (a liver function promoter as claimed) minerals, and soybean oil, and adding water to provide nutritional supplement to dogs and puppies or cats. '999 teaches that a protein source of whey protein (a glutathione promoter as claimed) and further supplemented with taurine and a **probiotic micro-organism** which beneficially effects the host by improving its intestinal microbial balance, such as lactic acid (col. 3, lines 25-40). '999 teach omega fatty acids such as **soybean oil** (claimed as intestinal function promoter and intestinal function promoter) and in Examples 1-2 (col. 3, lines 15-20). Soybean oil and vitamin (claimed as liver function promoter) has been shown to be at 1.7 percent by weight and 0.4% by weight respectively in Example 1 in column 4. The amount of soybean oil (a fatty acid with

profile and intestinal function promoter as claimed) is within the claimed range of between about 0.1% to 20%.

'999 does not characterize whey protein as glutathione promoter.

However, 571 or 412 correlate whey protein as glutathione promoters and teach glutathione promoters.

'571 or '412 teach a composition of whey protein concentrate (abstract).

'412 claims 1 and 2 teach compositions containing whey protein concentrate that promote glutathione as nutritional supplements to animals. The reference teaches immuno-enhancing effect maximized at 20%, see column 12, lines 48-58.

'571 teaches that a suitable source of whey protein is known by the trademark PROMOD, which contains whey protein and soy lecithin (col. 5, lines 34-41). Soy lecithin is taught by applicant in instant Example 2 to be an appropriate liver function promoter. '571 teaches that glutathione GSH promotion is a major function of the whey protein concentrate (w.p.c.) (col. 1, lines 30-37). '571 teaches the production of glutathione in the spleen, heart, liver is greater in mice fed with w.p.c, than mice fed with egg white protein (col. 4, lines 39-46).

The reference teaches use of about 18-28 gm of whey protein per 100 grams (18-20%.)., see claim 1.'571 teaches that the object of the invention is to provide a method for increasing the concentration level of glutathione in the organs and enhancing resistance to bacterial infection of mammals through the use of w.p.c, via oral administration (col. 10, lines 46-57). '571 also teaches inclusion of vitamins B1 and B2 with w.p.c. (claim 1-3, col. 11, lines 55-57).

The references disclosed above do not teach lipid assimilation, however, Simpson et al. disclose that vitamin E is a fat-soluble vitamin that is absorbed only with long chain fatty acids. A defect in either the absorption or digestion of lipid can therefore lead to deficiencies in this and other vitamins, due to their binding with unabsorbed fatty acids (Simpson, KW and Michel, KE. Micronutrient status in patients with gastrointestinal disease. Proceedings ACVIM, Denver, CO, pp. 651-653, 2001). Hence, a pet with low lipid digestibility is susceptible to several potential nutritional deficiencies, which can compromise its health. (See the entire articles of record).

A skilled artisan would thus have been motivated to provide a pet with an edible composition comprising liver function promoter in order to help in lipid assimilation which in turn helps in improving vitamin E absorption with a reasonable expectation of success based on the teachings of the disclosed references.

It would have been obvious to one of ordinary skill in the art to optimize the amount of liver function promoter such as glutathione promoters to obtain best possible results by doing experimental manipulations because '999 teaches soybean oil ( reads on both liver function promoter and intestinal function promoter) and vitamins in 1.7% and 0.4% amount (claimed as liver function promoter), as such it would have been within the purview of a skilled artisan to optimize the amount of glutathione, emulsifiers, taurine or any other liver function promoter to obtain best possible results and come to the claimed invention.

'999 does not teach pancreatic function promoter such as lipase. (a pancreatic function promoter)

367 claims in claim 1 a food supplement formulation of fish oil and lipase (the instant specification defines a pancreatic extract to be a lipase pg. 12, lines 1-3) (abstract, claim 1). The supplement of '367 improves bodily functions including fat metabolism, etc (col. 2, lines 26-30). The fish oil has specific fatty acid profile.

'999 and '367 references do not correlate the pancreatic function promoter (lipase) and intestinal mucosa function promoter such as probiotic microorganism with lipid absorption. Suzuki et al. disclose that bacterial or porcine Lipase with high or low fat diets optimizes fat absorption (see the entire article of record). It would have been obvious to the one of ordinary skilled in the art at the time the invention was made to incorporate pancreatic function promoter and intestinal mucosa function promoter in a feed composition and improve lipid absorption capacity of a pet animal with a reasonable expectation of success. WO '280 correlates the lipid absorption capacity with vitamin E absorption. As such, the pancreatic function promoter would have improved vitamin E absorption with the enhanced absorption of lipid in a pet animal in view of WO.

A skilled artisan would thus have been motivated to formulate a composition comprising liver function promoter, pancreatic function promoter and intestinal function promoter with a reasonable expectation of success in order to help increase lipid absorption and vitamin E absorption of a pet animal. Optimization of amounts would have been within the purview of a skilled artisan by doing experimental manipulations since the amounts depend on age, type of mammal, severity of vitamin deficiency, disease condition and condition of the mammal used, absent evidence to contrary.

***Response to Arguments***

8. Applicant's arguments with respect to claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 have been considered but are moot in view of the new ground(s) of rejection.

9. Claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 are rejected 35 U.S.C. 103(a) as being unpatentable over US Patent No. Fuchs et al WO 02/15719 ('719) in view of US 5,290,571 ('571) or US 5,451,412 ('412) and further in view of (Simpson, KW and Michel, KE. Micronutrient status in patients with gastrointestinal disease. Proceedings ACVIM, Denver, CO, pp. 651-653, 2001), (Suzuki et al. Gastroenterology 1999; 116:431-437 7), (W0 01/62280) and (USP 6,228,367).

'719 discloses a method of treatment which comprises administering an effective amount of the composition which contains whey protein to improve, promote, maintain intestinal function and mucins a patient or companion animal (abstract, claims 1-2 and 14-20, pg. 6 lines 5-10; pg. 12 lines 3-21).

Example 4 teaches a nutritional supplement comprising whey protein and probiotic bacteria. '719 teaches that the nature of whey protein and the fact that it is capable of being easily digested, the composition has a beneficial effect in patients with limited appetite due illness, surgery, chronic gastritis, etc (pg. 4, line 31-pg. 5, line 6), and that the addition of a probiotic micro-organism (pancreatic function promoter as claimed) provides the advantage of restoring the natural balance of the intestinal flora following antibiotic therapy (pg. 6, lines 7-10). Whey protein is taught by applicant to be

a fat transportation aid agent and carrier (instant spec pg. 10, 13-20). The amount of Whey protein is taught to be 4.8% and vitamins and minerals to at least 5% of RDA in example 1 on page 13 , '719 also teaches including a prebiotic (claim 13, pg. 5, lines 27-30). '719 teach including **taurine and** (claim 12, pg. 5, lines 18-25; pg. 6, lines 27-29), (claimed as liver function promoter in instant claims) . '719 teaches a lipid source including omega-3 fatty acids (abstract, claim 1). (claimed as intestinal function promoter in instant claims).

'719 teaches a nutritional supplement comprising whey protein and omega-3 fatty acids (abstract, claims 1-2). The reference teaches various amounts of polyunsaturated fatty acids including omega 3 fatty acid to be 15 to 30%, see page 8, lines 10-20. The reference teaches vitamins (claimed as liver function promoter in instant application), see page 9, lines 1-14.

'719 does not teach liver function promoter such as glutathione or glutathione promoters. However, 571 or 412 teach glutathione. '571 or '412 teach a composition of whey protein concentrate (abstract).

'412 claims 1 and 2 teach compositions containing whey protein concentrate that promote glutathione as nutritional supplements to animals. The reference teaches immuno-enhancing effect maximized at 20%, see column 12, lines 48-58.

'571 teaches that a suitable source of whey protein is known by the trademark PROMOD, which contains whey protein and soy lecithin (col. 5, lines 34-41). Soy lecithin is taught by applicant in instant Example 2 to be an appropriate liver function promoter. '571 teaches that glutathione GSH promotion is a major function of

the whey protein concentrate (w.p.c.) (col. 1, lines 30-37). '571 teaches the production of glutathione in the spleen, heart, liver is greater in mice fed with w.p.c, than mice fed with egg white protein (col. 4, lines 39-46).

The reference teaches use of about 18-28 gm of whey protein per 100 grams (18-20%.)., see claim 1.'571 teaches that the object of the invention is to provide a method for increasing the concentration level of glutathione in the organs and enhancing resistance to bacterial infection of mammals through the use of w.p.c, via oral administration (col. 10, lines 46-57). '571 also teaches inclusion of vitamins B1 and B2 with w.p.c. (claim 1-3, col. 11, lines 55-57).

It would have been obvious to one of ordinary skill in the art to optimize the amount of liver function promoter such as glutathione promoters to obtain best possible results by doing experimental manipulations because '719 teaches vitamin (claimed as liver function promoter) to be in supplied about 50 to 500% and in at least 5% of RDA, see example 1 on page 5, as such it would have been within the purview of a skilled artisan to optimize the amount of glutathione promoters such as whey protein within the claimed amount with a reasonable expectation of success.

The references disclosed above do not teach lipid assimilation, however, Simpson et al. disclose that vitamin E is a fat-soluble vitamin that is absorbed only with long chain fatty acids. A defect in either the absorption or digestion of lipid can therefore lead to deficiencies in this and other vitamins, due to their binding with unabsorbed fatty acids (Simpson, KW and Michel, KE. Micronutrient status in patients with gastrointestinal disease. Proceedings ACVIM, Denver, CO, pp. 651-653, 2001). Hence,

a pet with low lipid digestibility is susceptible to several potential nutritional deficiencies, which can compromise its health. (see the entire articles of record).

A skilled artisan would thus have been motivated to provide a pet with an edible composition comprising liver function promoter in order to help in lipid assimilation which in turn helps in improving vitamin E absorption with a reasonable expectation of success.

'719 does not teach pancreatic function promoter such as lipase.

'367 claims in claim 1 a food supplement formulation of fish oil and lipase (the instant specification defines a pancreatic extract to be a lipase pg. 12, lines 1-3) (abstract, claim 1). The supplement of '367 improves bodily functions including fat metabolism, etc (col. 2, lines 26-30). The fish oil has specific fatty acid profile.

'719 and '367 references do not correlate the pancreatic function promoter (lipase) and intestinal mucosa function promoter such as probiotic microorganism with lipid absorption.

Suzuki et al. disclose that bacterial or porcine Lipase with high or low fat diets optimize fat absorption (see the entire article of record). It would have been obvious to the one of ordinary skilled in the art at the time the invention was made to incorporate pancreatic function promoter and intestinal mucosa function promoter in a feed composition and improve lipid absorption capacity of a pet animal with a reasonable expectation of success. WO '280 correlates the lipid absorption capacity with vitamin E absorption. As such pancreatic function promoter would have improved vitamin E absorption with the enhanced absorption of lipid in a pet animal in view of WO.

A skilled artisan would thus have been motivated to formulate a composition comprising liver function promoter, pancreatic function promoter and intestinal function promoter with a reasonable expectation of success in order to help increase lipid absorption and vitamin E absorption of a pet animal. Optimization of amounts would have been within the purview of a skilled artisan by doing experimental manipulations since the amounts depend on age, type of mammal, severity of vitamin deficiency, disease condition and condition of the mammal used, absent evidence to contrary.

***Response to Arguments***

10. Applicant's arguments with respect to claims 35, 37, 39-41, 43, 45, 48-52 and 54-68 have been considered but are moot in view of the new ground(s) of rejection.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Snigdha Maewall whose telephone number is (571)-272-6197. The examiner can normally be reached on Monday to Friday; 8:30 a.m. to 5:00 p.m. EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick Krass can be reached on (571) 272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-0580. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status

information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Snigdha Maewall/

Examiner, Art Unit 1612

/Gollamudi S Kishore/

Primary Examiner, Art Unit 1612

| Notice of References Cited | Application/Control No. 10/509,951 | Applicant(s)/Patent Under Reexamination PEREZ-CAMRGO,GERARDO | |
|---|---|---|---|
| | Examiner Snigdha Maewall | Art Unit 1612 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Name | Classification |
|---|---|---|---|---|---|
| * | A | US-6,471,999 | 10-2002 | Couzy et al. | 426/2 |
| * | B | US-5,451,412 | 09-1995 | Bounous et al. | 424/535 |
| * | C | US-6,228,367 | 05-2001 | Watson, Tommy Stanley | 424/768 |
| | D | US- | | | |
| | E | US- | | | |
| | F | US- | | | |
| | G | US- | | | |
| | H | US- | | | |
| | I | US- | | | |
| | J | US- | | | |
| | K | US- | | | |
| | L | US- | | | |
| | M | US- | | | |

**FOREIGN PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Country | Name | Classification |
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| | N | | | | | |
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**NON-PATENT DOCUMENTS**

| * | | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |
|---|---|---|
| | U | |
| | V | |
| | W | |
| | X | |

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

# EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 10/509,951 | 10/04/2004 | **Gerardo Perez-Camargo** | 115808-509 | 3093 |

29157 7590 12/08/2009

K&L Gates LLP
P.O. Box 1135
CHICAGO, IL 60690

| EXAMINER | |
|---|---|
| MAEWALL, SNIGDHA | |
| ART UNIT | PAPER NUMBER |
| 1612 | |

| NOTIFICATION DATE | DELIVERY MODE |
|---|---|
| 12/08/2009 | ELECTRONIC |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

chicago.patents@klgates.com

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/509,951 | PEREZ-CAMARGO ET AL. | |
| | Examiner | Art Unit | |
| | Snigdha Maewall | 1612 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *20 August 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *35,45,48-52 and 57-64* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *35, 45, 48-52 and 57-64* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

### *Summary*

1. Receipt of Applicant's arguments/remarks and amended claims all filed on 08/20/09 is acknowledged.

Claims 1-34, 36-44, 46-47, 53-56 and 65-68 have been cancelled.

Claims 35, 45, 48-52 and 57-64 are pending in this application and claims **35, 45, 48-52 and 57-64** will be prosecuted on the merits.

### *Claim Rejections - 35 USC § 112*

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3 Claims 35, 45, 48-52 and 57-64 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 35 recites the limitation , acidifier which makes the claim indefinite. It is not clear which acidifier is utilized lactic acid or citric acid, the metes and bounds of claim is not defined. Claim 35 recite the limitation "fish oil" it is not clear which component is the applicant referring to, fish oil comprises EPA, DHA and other components. Specific recitation of components is requested.

Claims 45 and 57 recite the limitation "wherein the component has fatty acid profile selected to improve intestinal absorption; makes the claim indefinite. It is not clear which component is the Applicant referring to. There is no antecedent basis to claim. It is not clear how an acidifier can improve, maintain or promote cat's lipid absorption capacity and in turn improve or maintain absorption of vitamin E.

***Claim Rejections - 35 USC § 103***

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. Claims 35, 45, 48-52 and 57-64 are rejected 35 U.S.C. 103(a) as being unpatentable over (USP 6,471,999) in view of (USP 6,524,619) and further in view of (Simpson, KW and Michel, KE. Micronutrient status in patients with gastrointestinal disease, Proceedings ACVIM, Denver, CO, pp. 651-653, 2001, presented in IDS), (USP 6,228,367), (USP 6,610,007) and (W0 01/62280, presented in IDS).

'999 teach a pet milk powder as nutritional milk those results in reduced gastrointestinal intolerance (abstract). '999 teaches that the milk powder when administered in an effective amount with the nutritional composition reduces

gastrointestinal intolerance and that it may further comprise one or more lipid source, protein source, vitamins and minerals, and teaches a specific aspect which comprises lactose (of micro-organism origin), lactase, **taurine (a liver function promoter),** arginine and choline (claims 1-9; col. 2, lines 9-lines 26).

'999 teaches that a protein source of whey protein and further supplemented with **taurine and a probiotic micro-organism which beneficially effects the host by improving its intestinal microbial balance, such as lactic acid (col. 3, lines 25-40).** (Lactic acid reads on pancreatic function promoter, therefore, it is obvious that an acidifier such as lactic acid produced by probiotics help in improving intestinal balance, it is to be noted that probiotics are known to produce lactic acid and acetic acid, a pH modifying agent, which inhibit growth of bacteria, see instant specification page 8, paragraph 30).

'999 teaches chicory fibers, inulin, fructooligosaccharides with the probiotic micro-organism have a symbiotic relationship for promoting beneficial effects (col. 4, lines 9-14).

'999 teaches that the amount of nutritional composition is to be fed to a mammal each day depends on factors such as age, type of mammal (dogs and cats), and other nutritional sources (col. 4, lines 25-36). Examples 1 and 2 teach mixing the milk powder, galactosidase (lactase amino), **vitamins,** minerals, and soybean oil, and adding water to provide nutritional supplement to **dogs and puppies or cats.** '999 teaches that a protein source of whey protein and further supplemented with taurine and a **probiotic micro-organism** which beneficially effects the host by improving its intestinal microbial

balance, such as lactic acid (col. 3, lines 25-40). '999 teach omega fatty acids such as **soybean oil** (It is to be noted that fish oil is known in the art to comprise omega fatty acids such as EPA and DHA ,see USP 6,608,223) and in Examples 1-2 (col. 3, lines 15-20). Soybean oil and vitamin has been shown to be at 1.7 percent by weight and 0.4% by weight respectively in Example 1 in column 4. The amount of soybean oil (which comprises omega fatty acid reads on a fatty acid with profile as claimed in instant claim 45) is within the claimed range of between about 0.1% to 20%.

The references disclosed above do not teach correlation of taurine with lipid absorption and correlation of lipid absorption with vitamin E levels.

"619 teaches taurine enhances absorption of drug especially lipid soluble drugs and also teaches that bile salts are synthesized in the liver from cholesterol conjugated with taurine and within the gastrointestinal lumen these bile salts play an essential role in lipid absorption and fat transport, see column 22 and 23, lines 63-68 and 15-25.

Simpson et al. disclose that vitamin E is a fat-soluble vitamin that is absorbed only with long chain fatty acids. A defect in either the absorption or digestion of lipid can therefore lead to deficiencies in this and other vitamins, due to their binding with unabsorbed fatty acids (Simpson, KW and Michel, KE. Micronutrient status in patients with gastrointestinal disease. Proceedings ACVIM, Denver, CO, pp. 651-653, 2001, presented in IDS). Hence, a pet with low lipid digestibility is susceptible to several potential nutritional deficiencies, which can compromise its health. (See the entire articles of record).

A skilled artisan would thus have been motivated to provide a pet with an edible composition comprising liver function promoter such as taurine as taught by '999 in order to help in lipid absorption motivated by the teachings of '619 and would expect improvement in vitamin E absorption in light of the teachings of Simpson et al. which teaches that vitamin E deficiency occurs due to defect in lipid absorption.

It would have been obvious to one of ordinary skill in the art to optimize the amount of liver function promoter such as taurine to obtain best possible results by doing experimental manipulations because '999 teaches soybean oil ( reads on both liver function promoter and intestinal function promoter as taught in instant specification) and vitamins in 1.7% and 0.4% amount (claimed as liver function promoter in instant specification), as such it would have been within the purview of a skilled artisan to optimize the amount of the claimed liver function promoter, taurine to obtain best possible results and come to the claimed invention.

The teachings of references discussed above do not specifically teach fish oil in the composition.

'367 claims in claim 1 a food supplement formulation of **fish oil** and lipase (the instant specification defines a fish oil to be intestinal mucosa function promoter). The supplement of '367 improves bodily functions including fat metabolism, etc (col. 2, lines 26-30). The fish oil has specific fatty acid profile. It would have been obvious to one of ordinary skill in the art to utilize fish oil in the teachings of primary references in order to improve fat metabolism motivated by the teachings of '367. It would have been further obvious to one of ordinary to substitute fish oil in the teachings of the references

discussed above because '999 teaches inclusion of omega fatty acids in the composition and fish oil is known in the art to comprise omega fatty acids as is evident by USP 6,608,223.

The teachings of combined references taught above do not disclose correlation of fish oil (intestinal mucosa function promoter) with lipid absorption or vitamin E absorption.

'007 teaches fish oil enhances absorption of vitamin E tocopherol and vitamin A, retinol and teaches lipid digestion and absorption in rat model, see example 2 in column 11 and 12, lines 60-68 and 1-5 respectively.

Additionally, WO '280 correlates the lipid absorption capacity with vitamin E absorption. As such, vitamin E absorption with the enhanced absorption of lipid in a pet animal would have been obvious to one of ordinary skill in the art by administration of a composition comprising fish oil, (an intestinal mucosa function promoter) and taurine, (a liver function promoter) in light of teachings of '367 and '619, '007 and further in view of WO '280, one would have expected improvement in vitamin E absorption.

A skilled artisan would thus have been motivated to formulate a composition comprising liver function promoter, pancreatic function promoter and intestinal function promoter with a reasonable expectation of success in order to help increase lipid absorption and vitamin E absorption of a pet animal. Optimization of amounts would have been within the purview of a skilled artisan by doing experimental manipulations since the amounts depend on age, type of mammal, severity of vitamin deficiency, disease condition and condition of the mammal used, absent evidence to contrary.

6. Claims 35, 45, 48-52 and 57-64 are rejected 35 U.S.C. 103(a) as being unpatentable over US Patent No. Fuchs et al WO 02/15719 ('719) in view of (USP 6,524,619) and further in view of (Simpson, KW and Michel, KE. Micronutrient status in patients with gastrointestinal disease, Proceedings ACVIM, Denver, CO, pp. 651-653, 2001, presented in IDS), (USP 6,228,367), (USP 6,610,007) and (W0 01/62280, presented in IDS).

'719 discloses a method of treatment which comprises administering an effective amount of the composition which contains whey protein to improve, promote, maintain intestinal function and mucins a patient or **companion animal** (abstract, claims 1-2 and 14-20, pg. 6 lines 5-10; pg. 12 lines 3-21).

Example 4 teaches a nutritional supplement comprising whey protein and **probiotic bacteria**. (It is to be noted that probiotics are known to produce **lactic acid** and acetic acid, a pH modifying agent, which inhibit growth of bacteria, see instant specification page 8, paragraph 30).

719 teaches that the nature of whey protein and the fact that it is capable of being easily digested, the composition has a beneficial effect in patients with limited appetite due illness, surgery, chronic gastritis, etc (pg. 4, line 31-pg. 5, line 6), and that the addition of a probiotic micro-organism (pancreatic function promoter as claimed) provides the advantage of restoring the natural balance of the intestinal flora following antibiotic therapy (pg. 6, lines 7-10). Whey protein is taught by applicant to be a fat transportation aid agent and carrier (instant spec pg. 10, 13-20). The amount of Whey

protein is taught to be 4.8% and vitamins and minerals to at least 5% of RDA in example 1 on page 13 , '719 also teaches including a probiotic (claim 13, pg. 5, lines 27-30). '719 teach including **taurine and** (claim 12, pg. 5, lines 18-25; pg. 6, lines 27-29), (claimed as liver function promoter in instant claims). '719 teach a lipid source including **omega-3 fatty acids** (abstract, claim 1). (Claimed as intestinal function promoter in instant claims).

'719 teach a nutritional supplement comprising whey protein and omega-3 fatty acids (abstract, claims 1-2). The reference teaches various amounts of polyunsaturated fatty acids including omega 3 fatty acid to be 15 to 30%, see page 8, lines 10-20. The reference teaches vitamins (claimed as liver function promoter in instant application), see page 9, and lines 1-14.

The references disclosed above do not teach correlation of taurine with lipid absorption and correlation of lipid absorption with vitamin E levels.

"619 teaches taurine enhances absorption of drug especially lipid soluble drugs and also teaches that bile salts are synthesized in the liver from cholesterol conjugated with taurine and within the gastrointestinal lumen these bile salts play an essential role in lipid absorption and fat transport, see column 22 and 23, lines 63-68 and 15-25.

Simpson et al. disclose that vitamin E is a fat-soluble vitamin that is absorbed only with long chain fatty acids. A defect in either the absorption or digestion of lipid can therefore lead to deficiencies in this and other vitamins, due to their binding with unabsorbed fatty acids (Simpson, KW and Michel, KE. Micronutrient status in patients with gastrointestinal disease. Proceedings ACVIM, Denver, CO, pp. 651-653, 2001,

presented in IDS). Hence, a pet with low lipid digestibility is susceptible to several potential nutritional deficiencies, which can compromise its health. (See the entire articles of record).

A skilled artisan would thus have been motivated to provide a pet with an edible composition comprising liver function promoter such as taurine as taught by '719 in order to help in lipid absorption motivated by the teachings of '619 and would expect improvement in vitamin E absorption in light of the teachings of Simpson et al. which teaches that vitamin E deficiency occurs due to defect in lipid absorption.

'719 does not teach fish oil (claimed as intestinal mucosa function promoter).

'367 claims in claim 1 a food supplement formulation of **fish oil and** lipase (the instant specification defines a pancreatic extract to be a lipase pg. 12, lines 1-3) (abstract, claim 1). The supplement of '367 improves bodily functions including fat metabolism, etc (col. 2, lines 26-30). The fish oil has specific fatty acid profile. It would have been further obvious to one of ordinary to substitute fish oil in the teachings of the references discussed above because '999 teaches inclusion of omega fatty acids in the composition and fish oil is known in the art to comprise omega fatty acids as is evident by USP 6,608,223.

The teachings of combined references taught above do not disclose correlation of fish oil (intestinal mucosa function promoter) with lipid absorption or vitamin E absorption.

'0007 teaches fish oil enhances absorption of vitamin E tocopherol and vitamin A, retinol and teaches lipid digestion and absorption in rat model, see example 2 in column 11 and 12, lines 60-68 and 1-5 respectively.

Additionally, WO '280 correlates the lipid absorption capacity with vitamin E absorption. As such, vitamin E absorption with the enhanced absorption of lipid in a pet animal would have been obvious to one of ordinary skill in the art by administration of a composition comprising fish oil, (an intestinal mucosa function promoter) and taurine, (a liver function promoter) in light of teachings of '367, '619, '007 and further in view of WO '280, one would have expected improvement in vitamin E absorption.

A skilled artisan would thus have been motivated to formulate a composition comprising liver function promoter, pancreatic function promoter and intestinal function promoter with a reasonable expectation of success in order to help increase lipid absorption and vitamin E absorption of a pet animal. Optimization of amounts would have been within the purview of a skilled artisan by doing experimental manipulations since the amounts depend on age, type of mammal, severity of vitamin deficiency, disease condition and condition of the mammal used, absent evidence to contrary.

***Response to Arguments***

7. Applicant's arguments with respect to claims 35, 45, 48-52 and 57-64 have been considered but are moot in view of the new ground(s) of rejection.

8. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Snigdha Maewall whose telephone number is (571)-272-6197. The examiner can normally be reached on Monday to Friday; 8:30 a.m. to 5:00 p.m. EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Frederick Krass can be reached on (571) 272-0580. The fax phone number for the organization where this application or proceeding is assigned is 571-273-0580. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published

applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Snigdha Maewall/

Examiner, Art Unit 1612

/Gollamudi S Kishore/

Primary Examiner, Art Unit 1612

| Notice of References Cited | Application/Control No. 10/509,951 | Applicant(s)/Patent Under Reexamination PEREZ-CAMARGO ET AL. | |
|---|---|---|---|
| | Examiner Snigdha Maewall | Art Unit 1612 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Name | Classification |
|---|---|---|---|---|---|
| * | A | US-6,524,619 | 02-2003 | Pearson et al. | 424/472 |
| * | B | US-6,471,999 | 10-2002 | Couzy et al. | 426/2 |
| * | C | US-6,228,367 | 05-2001 | Watson, Tommy Stanley | 424/768 |
| * | D | US-6,610,007 | 08-2003 | Belson et al. | 600/146 |
| | E | US- | | | |
| | F | US- | | | |
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**FOREIGN PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Country | Name | Classification |
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**NON-PATENT DOCUMENTS**

| * | | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |
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*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

# EXHIBIT C

UNITED STATES PATENT AND TRADEMARK OFFICE

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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 10/509,951 | 10/04/2004 | Gerardo Perez-Camargo | 3714652.00509 | 3093 |

29157 7590 05/13/2010

K&L Gates LLP
P.O. Box 1135
CHICAGO, IL 60690

| EXAMINER | |
|---|---|
| MASWALL, SNIGDHA | |
| ART UNIT | PAPER NUMBER |
| 1612 | |

| NOTIFICATION DATE | DELIVERY MODE |
|---|---|
| 05/13/2010 | ELECTRONIC |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

chicago.patents@klgates.com

| Advisory Action Before the Filing of an Appeal Brief | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/509,951 | PEREZ-CAMARGO ET AL. | |
| | Examiner | Art Unit | |
| | Snigdha Maewall | 1612 | |

*--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

THE REPLY FILED 08 April 2010 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☒ The period for reply expires 3 months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.
   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.
   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☐ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: *35, 45, 48-52 and 57-64*.
   Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☒ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.
12. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
13. ☐ Other: _____.

/Snigdha Maewall/
Examiner, Art Unit 1612

/Gollamudi S. Kishore/
Primary Examiner, AU 1612

Continuation of 11. does NOT place the application in condition for allowance because:

Applicants contend that the submitted Declaration under 37 C.F.R. §1.132 demonstrates the unexpected results of administering an edible composition comprising an acidifier, taurine, and fish oil to a cat. As supported by the Declaration, a group of 20 cats with low fat digestibility (i.e., less than 80%) was fed diets to determine if, there was an improvement in fat digestibility in old cats fed different diets containing)combinations of pancreatic function promoters, liver function promoters, and intestinal mucosa function promoters, a "wet" diet (Diet A), a "dry" diet, (Diet B).

Applicants further add that the diets in the study contained a pancreatic function promoter (Diet A + citric acid), a liver function promoter (Diet A + taurine), an intestinal mucosa function promoter (Diet A + fish oil in the form of omega 3 oils), and a combination of the promoters (Diet C) were formulated and fed to cats using the procedure similar to that given in Example 1 of the above-identified patent application. The citric acid in the diets was in an amount of approximately 0.1% by weight. The taurine in the diets was in an amount of approximately 0.8% by weight. The fish oil in the diets was in an amount of approximately 3% by weight.

Applicants further add as supported by the Declaration, the control diets (Diet A and Diet B) showed a fat digestibility of about 61% and 63%, respectively, as shown in Figure 1 of the Declaration. There was no significant difference between fat digestibility of a wet diet and a dry diet. This confirms that the digestibility of wet and dry diets is substantially the same and that diet is not a factor in evaluating digestibility. Diet A + citric acid, Diet A + taurine, and Diet A + fish oil showed an increase in fat digestibility of 6.6%, 6.1% and 5.5% respectively when compared to the control diets. However, surprisingly, the combination of the three promoters showed a much more pronounced affect on fat digestibility. The combination (Diet C) showed an increase in fat digestibility of 17.5%.

Applicants state that in old cats with reduced fat digestibility (80%), the presence ofa single pancreatic function promoter (acidifier), a single liver function promoter (taurine), or a single intestinal mucosa function promoter (omega 3 oils) improved the level of fat digestibility (around 5.5 to 6.6%). However, none of these diets increased the level of fat digestibility above 80%, the level considered as normal. When the inventors provided the same old cats with a diet that contains a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils), the improvement in the level of fat digestibility is more dramatic (around 17.5%). Only with this diet did the old cats reach a level of fat digestibility that was considered normal (above 80%). This is a dramatic effect; not even in young healthy cats can fat digestibility be 100%. Moreover, no digestive system is 100% efficient (every meal produces some fecal content).

Applicants contend that as supported by the Declaration, the results are surprising and unexpected when the percentage of cats that showed an increase in fat digestibility is analyzed as shown in Figure 2 of the Declaration. The percent of cats that had an improved fat digestibility when administered thepromoters in combination was 90%, as compared to the 67% to 75% for the promoters alone. About 20% more cats will have increased fat digestibility if administered a combination or promoters than if administered one of the promoters alone. Thus, one critical discovery is that the number of cats that benefit from a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils) is much greater than the number of cats that benefit from a single promoter. Figure 2 shows that 90% of the cats improved their fat digestibility, versus only 75% when fed a diet with a single pancreatic function promoter (acidifier), 67 % with a single liver function promoter (Taurine), or 67% with a single intestinal mucosa function promoter (omega 3 oils),

Applicants arguments are not persuasive with respect to insufficient details in declaration as pointed out below:
First applicants show in figure 2, percent of cats that showed improvement, however no statistical data of percent of lipid absorption is shown in various cats. There is no fat digestion data presented so that comparison of cats with low or high absorption of fat with respect to consuming diet with only citric acid, or taurine or fish oil can be compared with cats who consumed diet with comblantion of the three ingredients such as citric acid, taurine and fish oil. The graph only shows percent of fat digestibility and percent of cats showing improvement, however, no comparative data for individual cat is shown in terms of lipid absorption.

Additionally, the term fat or lipid is a very broad and generic term which can encompass various forms such as fatty acids since fatty acids are building blocks of lipids, including neutral fat, over 70 different fatty acids have been isolated from various cells and tissues. Fatty acids which are building blocks of lipid may contain saturated and unsaturated bonds, some of the examples can be lauric acid, myristic acid or trans vaccenic acid etc. Simlarly fats can be acylglycerols or glycerides or di or triacylglycerides etc., see pages 189-190 of

Biochemistry book by Albert et al.(1970). Applicant claims lipid absorption capacity and provides declaration with fat absorption, therefore in light of the existence of several lipids and fats, it is not clear which specific group of fat or lipid absorption is the applicant referring to especially in the absence of statistical data to show fat absorption as pointed out above.

One aspect of the declaration is that the unexpected results shall commensurate with scope of claims, in the instant case, the unexpected results presented by applicants do not show if unexpected fat digestibility and will also exist with lower limits of taurine and fish oil. Instant claims also do not recite any specific amount for pancreatic function promoter and no specific acid such as citric acid is recited in instant claims, the claims recite acidifier generically. No data is presented to show unexpected results due to other pancreatic function promoters such as any acidifier. As such, the declaration is insufficient to overcome the rejection wherein it disclsoes taurine and fish oil to promote lipid digestibility.

Applicants aslo contend that the beneficial effects of the edible composition lead to an increase in fat digestibility in the cat that also correlates to an increase in the absorption capacity of Vitamin E by the cat. Applicants add that none of the cited references alone or in combination show the amount of liver function promoter to be from 0.1% to about 1% as claimed and the references do not show that composition improves otr maintains Vitamin E in a cat as claimed. Applicants similarly argue that Fuchs reference in combination with other references also do not teach the claimed range of taurine.

Applicants arguments are not persuasive, in response to applicants arguments that the claimed range of taurine is not taught by prior art, it is respectfully pointed out that '999 as discussed in the rejection does teach vitamins in the claimed range which has been described in instant specification as liver function promoter, therefore one of ordinary would have envisage utilizing another liver function promoter with an expectation to obtain similar results because '619 teaches that bile salts are synthesized in the liver from cholesterol conjugated with taurine and within the gastrointestinal lumen these bile salts play an essential role in lipid absorption and fat transport, see column 22 and 23, lines 63-68 and 15-25. (thus bile salt with taurine plays important role in fat absorption. Similarly, Fuchs also teaches vitamin in 5% amount and incombination with '619 and simpson, would have provided motivation to one of ordianry skill to utilize taurine as liver function promoter and thus improve vitamin E in pets. Besides, as discussed earlier due to insufficient evidnece in declaration , the rejections are maintained.

# EXHIBIT D

US006471999B2

(12) **United States Patent**
**Couzy et al.**

(10) **Patent No.: US 6,471,999 B2**
(45) **Date of Patent: Oct. 29, 2002**

(54) **MILK-BASED POWDER FOR PETS**

(75) Inventors: Francoise Couzy, Savigny (CH); Jean-Louis Leuba, deceased, late of Boussens (CH), by Christiane A. Leuba, Frederic Leuba, Aurelie Leuba, legal representatives

(73) Assignee: Nestec S.A., Vevey (CH)

( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/801,264

(22) Filed: Mar. 7, 2001

(65) **Prior Publication Data**

US 2002/0031571 A1 Mar. 14, 2002

**Related U.S. Application Data**

(63) Continuation of application No. PCT/EP99/06621, filed on Sep. 7, 1999.
(60) Provisional application No. 60/099,383, filed on Sep. 8, 1998.

(51) **Int. Cl.**[7] ..... **A23K 1/165**; A23K 1/18; A23C 9/00

(52) **U.S. Cl.** ..... **426/2**; 426/61; 426/588; 426/805

(58) **Field of Search** ..... 426/2, 61, 588, 426/805

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*Primary Examiner*—Chhaya D. Sayala
(74) *Attorney, Agent, or Firm*—Winston & Strawn

(57) **ABSTRACT**

A pet milk powder which, when reconstituted and fed to a pet as a nutritional milk, results in reduced gastrointestinal intolerance. This powder is a cow's milk powder that contains lactose and to which is added a lactase, preferably one that is active under acidic conditions, such as a β-galactosidase.

**10 Claims, No Drawings**

# MILK-BASED POWDER FOR PETS

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of the U.S. national phase designation of international application PCT/EP99/06621, filed Sep. 7, 1999, the content of which is expressly incorporated herein by reference thereto, which claims the benefit of Provisional application Ser. No. 60/099,383, filed Sep. 8, 1998.

## TECHNICAL FIELD

This invention relates to a milk-based powder that may be reconstituted to provide a milk-based nutritional composition for pets and especially for young pets.

## BACKGROUND ART

Many pet owners, especially owners of young pets, feed cow's milk or cow's milk based compositions to their pets since cow's milk is an excellent source of nutrition. Further, in cases where very young pets are unable to obtain milk from their mothers, cow's milk or compositions based upon cow's milk may be the only source of nutrition for the young animal.

Unfortunately, the feeding of cow's milk to pet mammals may result in gastrointestinal intolerance. This manifests itself in a variety of intestinal symptoms which include bloating, distension, cramp, flatulence, lower faecal consistency and, in severe cases, diarrhoea. Lower faecal consistency and diarrhoea are particularly well known symptoms (Mundt, H-C. and Meyer, H.; 1989, Waltham Symposium 7: Nutrition of the Dog and Cat, Cambridge University Press, pages 267–274). The cause of the gastrointestinal intolerance is attributed to the lactose in cow's milk.

Removal of lactose from cow's milk for human applications is well known. This is usually done by micro- or ultra-filtration or enzymatic treatment, or both, of liquid milk or whey solutions. Further, milk or whey powders which are low in lactose, or lactose free, are commercially available and may be fed to pets, but these powders are generally too expensive for commercial use in pet products. For pets, a possible solution to the problem is described in European patent application 0259713. Here the lactose in the composition is reduced by reducing the content of milk powder in the composition to below about 60% by weight. In order to make up for the reduction in protein, lactose-reduced or lactose-free milk proteins are then added to the composition. In this way, the lactose content of the composition may be reduced to below about 30% by weight, but this requires the addition of large amounts of lactose-reduced or lactose-free milk proteins which increases the cost.

Muodt and Meyer (supra) suggest that another solution to this problem is to hydrolyze the lactose using enzymes prior to producing the pet milk powder. This is an acceptable solution when milk is freely and inexpensively available in liquid form, but it is not a feasible solution when the milk ingredient is available in powdered form; which is commonly the case.

Therefore there is still a need for a cow's milk-based powder which may be reconstituted to provide a milk-based nutritional composition, which is relatively simple to prepare and relatively inexpensive.

## SUMMARY OF THE INVENTION

Accordingly, in one aspect, this invention provides a pet milk powder comprising a cow's milk powder which contains lactose, and a lactase.



It is surprisingly found that the simple addition of lactase to milk powder is able to avoid or significantly reduce the gastrointestinal problems associated with the consumption of lactose. This is despite the fact that the milk composition produced by reconstituting the milk powder may be consumed immediately after reconstitution; that is before the lactase has had the time to degrade the lactose in the milk powder.

Preferably, the lactase is a β-galactosidase; more preferably one from micro-organism origin. A β-galactosidase which is active at an acidic pH is particularly preferred.

The milk powder may further comprise one or more of a lipid source, protein source, vitamins and minerals.

In another aspect, this invention provides a milk powder for cats, the powder comprising a cow's milk powder which contains lactose, a lactase, taurine, arginine and choline.

In a yet further aspect, this invention provides a milk powder for dogs, the powder comprising a cow's milk powder which contains lactose, a lactase, and choline.

In a further aspect, this invention provides a method for reducing the symptoms of gastrointestinal intolerance in a mammalian pet after consumption of a nutritional composition based on cow's milk, the method comprising administering to the pet an effective amount of a lactase in combination with the nutritional composition.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Preferred embodiments of the invention are now described by way of example only.

The invention concerns a milk-based powder which may be reconstituted with water to provide a nutritional milk for pets which has reduced gastrointestinal intolerance.

The milk-based powder contains cow's milk powder and a lactase. The cow's milk powder may be any suitable milk powder which is based upon cow's milk; for example skimmed milk powder and whole milk powder. Further, milk powders produced from standardized milk-based solutions may be used. If desired, the cow's milk powder may contain additives such as vitamins, minerals, protein, lipids, and the like. The lactose content of the milk powder is not critical to the invention. Of course, if cow's milk powders having low lactose contents are readily and inexpensively available, they may be advantageously used.

The lactase may be any suitable lactase which is generally recognized as safe. β-galactosidases are preferred; especially β-galactosidases of microbial origin. Since conditions in the gastrointestinal tract are acidic, a lactase which remains active under acidic conditions is preferred. It is also possible to use lactases which are active under neutral or basic conditions. In these cases, however, it may be useful to include an alkali in the milk-based powder which slows the pH drop in the gastrointestinal tract.

An enzyme which is particularly suitable is a β-galactosidase which may be obtained from Amano Enzyme USA Co Ltd of Lombard, IL, USA. The enzyme is available under the name "Lactase Amano". The enzyme is obtained from *Aspergillus oryzae* and has an optimum pH of about 4.8 when lactose is the substrate. The enzyme has an activity of more than 50000 units/g at optimum pH. The enzyme is generally recognized as safe and is food grade.

The amount of the lactase to be added will depend upon various factors such as the lactose content of the cow's milk powder and the activity of the enzyme. The useful amount may be readily determined by a skilled person. Ordinarily,

the lactase may be added to provide about 25 UI/100 g to about 200 UI/100 g powder; for example about 50 UI/100 g to about 125 UI/100 g powder. The unit, UI, indicates the amount of enzyme which produces 1 micromole of o-Nitrophenol per minute at 30° C. when 3.0 ml of a solution which contains 200 mg of o-Nitrophenol-β-galactopyranoside per 100 ml of 0.1 M McIlvaine buffer, pH 4.5; is added to 1.0 ml of diluted enzyme solution. The reaction is stopped after 10 minutes.

For an enzyme which has an activity of about 50 UI/100 g to about 125 UI/100 g powder, the lactase may comprise about 0.05% to about 0.4% by weight of the milk-based powder; and preferable from about 0.15% to about 0.25% by weight.

If it is desired to make the milk-based powder more nutritionally complete, other nutritional components may be added to the powder. For example, a lipid source may be added to the milk-based powder. Any suitable lipid source may be used; for example vegetable oils such as soybean oil, sunflower oil, safflower oil, corn oil, peanut oil, and rapeseed oil, or animal fats such as milk fats and tallow. In general, the lipid source used will be selected on the basis of nutritional value, cost and palatability considerations.

It is also possible to add further protein and amino acids sources. For example, whey protein powders may be added to the milk-based powder. Similarly, the milk-based powder may be supplemented with free amino acids which are required by the mammal for complete nutrition. For example, for milk-based powder intended for kittens, the powder may be supplemented with taurine or arginine, or both.

The milk-based powder may also contain vitamins and minerals. It is particularly preferred to include a source of calcium; for example dicalcium phosphate.

The milk-based powder may also include a probiotic micro-organism. A probiotic micro-organism is a micro-organism which beneficially affects a host by improving its intestinal microbial balance (Fuller, R; 1989; *J. Applied Bacteriology*, 66: 365–378). In general, probiotic micro-organisms produce organic acids such as lactic acid and acetic acid which inhibit the growth of pathogenic bacteria. Examples of suitable probiotic micro-organisms include yeasts such as *Saccharomyces*, *Debaromyces*, *Candida*, *Pichia* and *Torulopsis*, moulds such as *Aspergillus*, *Rhizopus*, *Mucor*, and *Penicillium* and *Torulopsis* and bacteria such as the genera *Bifidobacterium*, *Bacteroides*, *Clostridium*, *Fusobacterium*, *Melissococcus*, *Propionibacterium*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Staphylococcus*, *Peptostrepococcus*, *Bacillus*, *Pediococcus*, *Micrococcus*, *Leuconostoc*, *Weissella*, *Aerococcus*, *Oenococcus* and *Lactobacillus*. Specific examples of suitable probiotic micro-organisms are: *Saccharomyces cerevisease*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Enterococcus faecium*, *Enterococcus faecalis*, *Lactobacillus acidophilus*, *Lactobacillus alimentarius*, *Lactobacillus casei* subsp. *casei*, *Lactobacillus casei Shirota*, *Lactobacillus curvatus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus farciminus*, *Lactobacillus gasseri*, *Lactobacillus helveticus*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus* (Lactobacillus GG), *Lactobacillus sake*, *Lactococcus lactis*, *Micrococcus varians*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Pediococcus halophilus*, *Streptococcus faecalis*, *Streptococcus thermophilus*, *Staphylococcus carnosus*, and *Staphylococcus xylosus*. The probiotic micro-organisms are preferably in powdered, dried form; especially in spore form for micro-organisms which form spores. Further, if desired, the probiotic micro-organism may be encapsulated to further increase the probability of survival; for example in a sugar matrix, fat matrix or polysaccharide matrix.

Further, the milk-based powder may also include a source of a fermentable soluble fiber, for example, chicory fibers, inulin, fructooligosaccharides, and the like. Preferably the fermentable soluble fibre selected is a substrate for the probiotic micro-organism selected, or such that the fermentable soluble fiber and probiotic micro-organism form a symbiotic relationship for promoting beneficial effects.

It is of course possible that vitamins, minerals, amino acids and a lipid source may have been used in the preparation of the cow's milk powder. In this case, less or none of these ingredients need be added.

The milk-based powder may be manufactured by dry mixing the cow's milk powder, the lactase, and any other ingredients. If a lipid source is added, it is preferably mixed in last. Any suitable mixing apparatus may be used. The milk-based powder is then packed into suitable packages.

The amount of the nutritional composition to be fed to a mammal each day will depend upon factors such as the mammal's age, the type of mammal, and other sources of nutrition. In general, the nutritional composition may be used in much the same way and in the same amounts as milk is used. For example, for medium and large dogs, up to about 250 ml of the nutritional composition per day may be fed to the dog. For smaller dogs, up to about 125 ml of the nutritional composition per day may be fed to the dog. Similar values may be readily determined for cats and other mammals.

## EXAMPLES

By way of illustration, specific examples of the invention are now described.

Example 1

A milk-based powder for dogs is prepared by dry mixing together whole milk powder, β-galactosidase ("Lactase Amano"), vitamins, minerals and soybean oil. The composition of the powder is as follows:

| Ingredient | Percent by Weight |
|---|---|
| Milk powder | 96.2 |
| Soybean oil | 1.7 |
| Dicalcium phosphate | 1.1 |
| Choline | 0.4 |
| β-galactosidase | 0.2 |
| Vitamins, Minerals | 0.4 |

The milk-based powder has a lactose content of about 33% by weight. The milk powder is added to tap water and is rapidly reconstituted to provide a milk-based nutritional composition. The nutritional composition is highly palatable to puppies and dogs.

Example 2

A milk-based powder for cats is prepared by dry mixing together whole milk powder, β-galactosidase, arginine, taurine, vitamins, minerals and soybean oil. The composition of the powder is as follows:

| Ingredient | Percent by Weight |
|---|---|
| Milk powder | 97.1 |
| Dicalcium phosphate | 1.5 |
| Choline | 0.4 |
| Arginine | 0.4 |
| β-galactosidase | 0.2 |
| Soybean oil | 0.05 |
| Vitamins, Minerals | 0.35 |

The milk-based powder has a lactose content of about 33% by weight. The milk powder is added to tap water and is rapidly reconstituted to provide a milk-based nutritional composition. The nutritional composition is highly palatable to kittens and cats.

Example 3

Seven beagle dogs 5 to 12 years are used in a trial. Each dog is separately housed in a cage. The dogs have access to a dry diet *ad libitum*.

In the first part of the trial, the dogs are fed a milk reconstituted from a full fat milk powder for a period of 7 days. The milk contains vitamins and minerals. The milk is reconstituted immediately before serving by adding cold tap water to the fill fat milk powder. Food consumption, liquid consumption and faecal consistency are monitored.

In the second part of the trial, the dogs are fed a nutrition composition reconstituted from the milk-based powder of example 1 for a period of 7 days. The nutrition composition is reconstituted immediately before serving by adding cold tap water to the milk-based powder. Food consumption, liquid consumption and faecal consistency are monitored.

In both parts of the trial, each dog is fed 900 g per day of the milk or nutritional composition. The milk or nutritional composition is available from 9 a.m. to 3 p.m. and is the only liquid source during this period. In general, the entire amount of liquid is consumed rapidly. From 3 p.m. to 9 a.m., the dogs have free access to water.

| Food | Percentage of stool having loose stool consistency | Percentage of stools being diarrhoeic |
|---|---|---|
| Milk | 36 | 19 |
| nutritional composition of example 1 | 12 | 7 |

The nutritional composition offers a significant improvement even at this high level of consumption.

Example 4

Seven cats aged 5 to 12 years are used in a trial. Each cat is separately housed in a cage. The cats have access to a fish-based dry diet *ad libitum*.

In the first part of the trial, the cats are fed a milk reconstituted from a full fat milk powder for a period of 7 days. The milk contains vitamins and minerals. The milk is reconstituted immediately before serving by adding cold tap water to the full fat milk powder. Food consumption, liquid consumption and faecal consistency are monitored.

In the second part of the trial, the cats are fed a nutrition composition reconstituted from the milk-based powder of example 2 for a period of 7 days. The nutrition composition is reconstituted immediately before serving by adding cold tap water to the milk-based powder. Food consumption, liquid consumption and faecal consistency are monitored.

In both parts of the trial, each cat is presented with 180 g per day of the milk or nutritional composition. The milk or nutritional composition is available from 3:00 p.m. to 9 a.m. and is the only liquid source during this period. From 9 a.m. to 3 p.m., the cats have free access to water.

| Food | Percentage of stool having loose stool consistency | Percentage of stools being diarrhoeic |
|---|---|---|
| Milk | 42 | 37 |
| nutritional composition of example 2 | 20 | 0 |

The nutritional composition offers a very significant improvement. No significant change in consumption between the milk and nutritional composition is noticed. Hence palatability is unaffected by the addition of the enzyme.

Example 5

A milk-based powder is prepared using a β-galactosidase enzyme obtained from Novo Nordisk A/S of Bagsvaerd, Denmark and sold under the name Lactozym. The powder is substantially identical to the powder of example 1 except that this different enzyme is used. The enzyme is optimally active under basic conditions.

When fed to beagle dogs, the milk-based powder has substantially the same properties as the powder of example 1.

What is claimed is:

**1**. A method for reducing the symptoms of gastrointestinal intolerance in pets who ingest a reconstituted pet milk powder comprising a cow's milk containing lactose, which method comprises administering to the pet a lactase in an amount sufficient to reduce symptoms of gastrointestinal intolerance in the pet, wherein the lactase is administered as an ingredient of the pet milk composition that is to be reconstituted.

**2**. The method according to claim **1** in which the lactase is present in the pet milk powder in an amount of between about 0.05 to 0.4% by weight of the powder.

**3**. The method according to claim **1** in which the lactase is one that has optimum activity under acidic conditions.

**4**. The method according to claim **1** in which the lactase is a β-galactosidase.

**5**. The method according to claim **4** in which β-galactosidase is of microbial origin.

**6**. The method according to claim **1** in which the lactase provides about 75 UI/100g to about 125 UI/100g of powder.

**7**. The method according to claim **1** which further comprises at least one of a lipid source, a protein source, one or more vitamins or one or more minerals.

**8**. The method according to claim **1** which further comprises a calcium source.

**9**. The method of claim **1** specifically formulated for cats, the powder containing taurine and choline.

**10**. The method of claim **1** specifically formulated for dogs, the powder containing choline.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

# CERTIFICATE OF CORRECTION

PATENT NO. : 6,471,999 B2
DATED : October 29, 2002
INVENTOR(S) : Couzy et al.



It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page.
Item [75], Inventors, change "**Francoise Couzy**, Savigny (CH)" to -- **Francois Couzy**, La Croix sur Lutry (CH) --.
Item [56], **References Cited**, OTHER PUBLICATIONS, change "al. (eds) 2nd edu." to -- al. (eds) 2nd edn. --.

Signed and Sealed this

Twenty-eighth Day of January, 2003

JAMES E. ROGAN
*Director of the United States Patent and Trademark Office*

# EXHIBIT E

US006524619B2

(12) **United States Patent**
**Pearson et al.**

(10) **Patent No.: US 6,524,619 B2**
(45) **Date of Patent: Feb. 25, 2003**

(54) **DOSAGE FORMS USEFUL FOR MODIFYING CONDITIONS AND FUNCTIONS ASSOCIATED WITH HEARING LOSS AND/OR TINNITUS**

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( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/765,974

(22) Filed: **Jan. 19, 2001**

(65) **Prior Publication Data**

US 2002/0061870 A1 May 23, 2002

**Related U.S. Application Data**

(60) Provisional application No. 60/178,487, filed on Jan. 27, 2000.

(51) **Int. Cl.**[7] ..... **A61K 9/20**; A61K 9/24; A61K 9/22

(52) **U.S. Cl.** ..... 424/472; 424/464; 424/468

(58) **Field of Search** ..... 424/468, 472, 424/464, 489, 473, 457

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(57) **ABSTRACT**

The invention defines interdependent biofactors and biomolecules, and clinically useful formulations that are comprised of them. The active agents are demonstrated to be complementary in their physiologic functions especially as these relate to the quenching of free radicals and to the support of endothelial physiology, the reduction of hyperinsulinemia and improvements in vascular health. The active components of the invention are selected for inclusion in precise combinations specifically because they improve these various conditions and physiological functions, and by so doing reduce a variety of risks associated with hearing loss and tinnitus. The resulting enhancement of general systemic vascular health, improvement in local VIII[th] nerve vascular health, modulation of conditions surrounding blood fluid dynamics, the consequences of hyperinsulinemia, and improvements in free radical defenses, all reduce the potential for cochlear hair cell death and VIII[th] nerve atrophy, and the hearing loss and possible deafness that accompany them.

**14 Claims, No Drawings**

# DOSAGE FORMS USEFUL FOR MODIFYING CONDITIONS AND FUNCTIONS ASSOCIATED WITH HEARING LOSS AND/OR TINNITUS

## CROSS REFERENCE TO RELATED APPLICATION

This application is related to United States Provisional Patent Application No. 60/178,487, filed Jan. 27, 2000, and claims all benefits legally available therefrom. Provisional Patent Application No. 60/178,487 is hereby incorporated by reference for all purposes capable of being served thereby.

## BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention is in the field of pharmacology and relates specifically to the improvement of clinical conditions associated with symptomatic or presymptomatic hearing loss and/or tinnitus and the reduction of risks associated with their onset.

2. Description of the Prior Art

### Pertinent Anatomy of the Ear

The ear of humans consists of three parts: the outer, middle and inner ear. The outer ear consists of the external ear and the auditory canal. The external ear modifies sound waves and the air-filled auditory canal conducts the sound waves to the middle ear, which consists of the tympanic membrane, or eardrum; the eustachian tube; and three tiny bones called the hammer, anvil, and stirrup. Membranes and bone surround the middle ear with the eustachian tube connecting it to the pharynx, equalizing the air pressure between the middle ear and the atmosphere.

Within the middle ear, sound first vibrates the tympanic membrane, which in turn vibrates the hammer, the anvil, and the stirrup. These bones transmit vibrations from the tympanic membrane to a much smaller membrane, the oval window. The oval window covers the opening of the inner ear, in which sound vibrations are transmitted through fluid. The fluid-filled hollow bones of the inner ear form the spiral-shaped cochlea and the vestibular apparatus where vibrations are translated into neural signals.

The senses of hearing and equilibrium depend on sensory receptors called hair cells located on the basilar membrane of the cochlea. These hair cells can detect motions of atomic dimensions and respond more than 100,000 times a second. Biophysical studies suggest that mechanical forces control the opening and closing of transduction channels by acting through elastic components in each hair cell's mechanoreceptive hair bundle. Other ion channels, as well as the mechanical and hydrodynamic properties of hair bundles, tune individual hair cells to particular frequencies of stimulation.

Even though well characterized at a biophysical level, the mechanical transduction mechanism of hair cells is still not completely understood in molecular terms. This discrepancy is in part due to the extreme scarcity of hair cells; instead of the millions or even hundreds of millions of receptor cells that the olfactory and visual systems possess, only a few tens of thousands of hair cells are found in the internal ears of most vertebrate species. The small number of hair cells and the direct transduction mechanism has greatly impeded molecular biological and biochemical characterization. Molecular description of hair-cell transduction has consequentially lagged behind description of vision and olfaction.



A comprehensive model for hair-cell transduction has emerged. Residing in the mechanoreceptive organelle of a hair cell, the hair bundle (the transduction apparatus) consists of at least three components: the transduction channel, a mechanically gated ion channel; the tip link, an extracellular filament that transmits force to the channel's gate; and the adaptation motor, a mechanism that maintains an optimal tension in the tip link so that the channel can respond to displacements of atomic dimensions.

The tip link appears to be the anatomical correlate of a gating spring, an elastic element through which stimulus energy can affect the transduction channel. A cluster of myosin molecules constitutes the adaptation motor. Hair cells express a variety of myosin isozymes.

The specialized innervation of hair cells makes the restoration of hearing potentially a practical form of neural-replacement therapy. Hair cells lack axons and dendrites; instead, the basolateral surfaces of these cells make afferent synaptic contacts with VIIIth nerve terminals and receive efferent contacts from neurons in the brainstem. When hair cells are destroyed, this innervation often remains intact; indeed, the integrity of the afferent innervation underlies the success of cochlear prosthetics. If hair cells can be successfully regenerated, it follows that their re-innervation may be possible. In contrast, in other proposed neural-replacement therapies, transplanted neurons are called upon to extend their axons substantial distances in order to make appropriate connections. It is questionable whether such axiogenesis is possible in the adult brain or spinal cord.

Axons in the cochlear component of the VIIIth VIIIth nerve project to each of the three cochlear nuclei, an orderly representation of stimulus frequency is preserved at each subsequent level of the ascending pathway. Extensive decussation occurs at the pontine and midbrain levels. Then, via the superior olivary nuclei process, information is transmitted to an auditory spatial map in the inferior colliculus and finally via the medial geniculate nucleus to the temporal cerebral cortex.

### Pertinent Physiology of the Ear

The defining event in the hearing process is the transduction of mechanical stimuli into electrical signals by hair cells, the sensory receptors of the internal ear. Stimulation results in the rapid opening of ionic channels in the mechanically sensitive organelles of these cells, their hair bundles. These transduction channels, which are non-selectively permeable, are directly excited by hair-bundle displacement. Hair cells are selectively responsive to particular frequencies of stimulation, due to both the mechanical properties of their hair bundles and because of an ensemble of ionic channels that constitutes an electrical resonator.

The unique structural feature of the hair cell is the hair bundle, an assemblage of microscopic processes protruding from the cell's top or apical surface. Each of these processes, which are termed stereocilia, consists of a straight rod of fasciculated actin filaments surrounded by a membranous tube. Because the microfilaments are extensively cross-bridged, each stereocilium behaves as a rigid rod. When mechanically disturbed, it remains relatively straight along its length but pivots about a flexible basal insertion. When the fluid moves in response to sound, the force of viscous drag bends the bundles, thereby initiating a response.

At any instant, each transduction channel at a stereocilium's tip may be either closed or open. The relative values of the rate constants for channel opening and closing determine the fraction of the transduction channels open in the undis-

turbed steady state. When the hair bundle is deflected with a positive stimulus, the values of the rate constants are altered; the opening rate constant is larger and the closing rate constant smaller than the original values. The new steady-state transduction current is therefore greater and the cell is depolarized. Pushing the hair bundle in the opposite direction has a contrary effect on the rate constants, culminating in a hyperpolarizing response.

When the hair bundle is deflected, transduction channels open and positive ions, largely $K^+$, enter the cell. The depolarization evoked by this transduction current activates voltage-sensitive $Ca^{2+}$ channels. As $Ca^{2+}$ ions flow into the cell they augment depolarization and raise the intracellular concentration of $Ca^{2+}$, especially the local concentration just beneath the surface membrane. Elevated $Ca^{2+}$ concentrations activate $Ca^{2+}$-sensitive $K^+$channels. As $K^+$ exits through these pores it initiates membrane repolarization and diminishes the activation of $Ca^{2+}$ channels. The fluid bathing the apical surface of a hair cell characteristically has a much higher $K^+$ concentration than that contacting the basolateral surface; as a consequence, $K^+$ can both enter and leave the cell passively. Once the membrane potential becomes more negative than its steady-state value, intracellular $Ca^{2+}$ concentration is reduced by sequestration of the ion within cytosolic organelles and by extrusion through $Mg^{++}$ cofactored, ATPase-fueled ion pumps. The $Ca^{2+}$ -sensitive $K^+$ channels have now closed and the hair cell returns to its initial condition.

## Hearing Loss and/or Tinnitus—The Disease

### Prevalence and Socioeconomic Impact

Society is awakening only slowly to the cost of acoustic trauma in both industrial and recreational settings. Within the next decade or two, the Walkman® generation will find itself unexpectedly interested in auditory pathophysiology. Varying degrees of deafness affect about 30 million Americans and cost the nation over $56 billion annually (Dana Alliance for Brain Initiatives, 1996).

In a population-based study of 3,753 residents in Beaver Dam, Wis., the prevalence of hearing loss in adults aged 48–92 years, was 45.9%. The average age of participants was 65.8 years. The hearing loss increased with age and was greater for men than women. The prevalence of hearing loss is a striking 95 percent in the 80+ year age group, which translates into an increasing problem in nursing homes.

Hearing loss is a growing problem in occupational health, including the military wherein about one-third had hearing loss by the end of basic training. Hearing loss is evident even when precautions are taken.

Tinnitus is also increasingly common (about 15% of adults#17923]) and is often an early indicator of existing or future hearing loss. This hearing loss is ordinarily permanent, for cochlear hair cells are not replaced by mitotic turnover. Although cochlear prostheses have now restored partial hearing to some 15,000 deaf individuals worldwide and researchers continue to seek a means of overcoming deafness by the replacement of hair cells, improved prevention represents the most reasonable present approach.

### Pathophysiology of Hearing Loss and/or Tinnitus

Hearing loss may occur acutely due to hair cell trauma from excessive $Ca^{2+}$ signaling and generation of reactive oxygen species (ROS) from noise exposure. Chronic progressive hearing loss is often associated with labyrinthine ischemia from either hematologic disturbances (e.g., increased blood viscosity, decreased red blood cell deformability) or from non-hematologic vasoconstriction due to progressive vascular endothelial dysfunction. Chronic neurosensory hearing loss accompanies aging and is more common in the diabetic population.

#### I. Acute Hair Cell Trauma

Noise-induced trauma to the hair cells is sound-intensity dependent and can lead to hair cell death. Since mitotic hair cell replacement does not occur, traumatic hearing loss is permanent. The pathophysiology of traumatic hair cell loss is multivariable. Hypoxia, excessive $Ca^{2+}$ signaling, vasoconstriction, intracellular energy exhaustion, ROS and ROS and excitotoxicity, each contribute individually and as a detrimental synergistic composite.

As previously stated, when the hair bundle is deflected transduction channels open and activate voltage-sensitive $Ca^{2+}$ channels. $Ca^{2+}$ inflow into the cell causes depolarization. At the same time, however, as the intracellular concentration of $Ca^{2+}$ rises —especially its local concentration just beneath the surface membrane of the hair cell—these high $Ca^{2+}$ concentrations activate $Ca^{2+}$sensitive $K^+$ outflow channels; an energy requiring activity supported by $Mg^{2+}$-cofactored ATPase. As $K^+$ exits, membrane repolarization begins and closes the $Ca^{2+}$ channels. Once the membrane potential is somewhat more negative than its steady-state value, intracellular $Ca^{2+}$ concentration is further reduced by sequestration of the ion within cytosolic organelles and by its continuing extrusion through $Mg^{++}$ cofactored, ATPase-fueled ion pumps.

Shear stress is increased by vasoconstriction (inter alia) and increased shear stress raises $Ca^{2+}$ channel permeability as much as ten or twelve fold. Since $Ca^{2+}$ influx augments the endothelin-1-induced vasoconstrictive effect of pooled intracellular $Ca^2$, a "circle-in-a-spiral" vasoconstrictive effect occurs. The components of this patent, operating in concert, untie this metaphorically tangled biologic shoelace.

This complex physiology of hearing can become overwhelmed by high intensity and/or prolonged sound. Energy for hearing is supplied by mitochondria which are only 95% efficient in their use of oxygen; 5% of oxygen ends up as potentially damaging ROS. Furthermore, excessive hearing demands result in excessive oxygen requirements that, in turn, lead to hair cell hypoxia. This situation is further exaggerated by local vasoconstriction resulting from ROS-induced, endothelial cell dysfunction. Excessive energy requirements exhaust the ability of the cells to extrude $Ca^{2+}$; as a result, $Ca^{2+}$ pools intracellularly and as long as the intense sound continues it continues to pour into the cell (see above). Excess intracellular $Ca^{2+}$ leads to the production of endothelin-1 (ET-1), which has a prolonged and intense vasoconstrictor action, exaggerating the effect of hypoxia and delaying hair cell recovery. Of course, the hair cell may go on to die, not to be replaced.

Enzymes involved in maintaining glutathione (gamma-glutamyl-cysteinyl-glycine (GSH in the reduced state) protect hair cells from ROS-induced damage. This suggests that agents that protect or augment the GSH system in the cochlea may be protective against noise-induced hearing loss.

Cochlear ischemia (which is discussed at length later) and acoustic trauma result in an immediate hearing loss accompanied by the complete disruption of the terminal dendrites of primary auditory neurons postsynaptic to the sensory inner hair cells (IHCs). This synaptic uncoupling is due to an acute glutamate (IHC neurotransmitter) excitotoxicity pro-

cess. However, some repair is possible: twenty-four hours after an excitotoxic injury the inner hair cells may be contacted by postsynaptic dendrites and cochlear function may recover partially. Neo-synaptogenesis may be complete 5 days post exposure and this type of functional recovery probably accounts for most restored hearing after temporary losses due to excitotoxic-related pathologies.

### Cellular Defenses Available for Acute, Sound-induced Hair Cell Trauma

#### 1. Regulate the Passive Transfer of $Ca^{2+}$into the Hair Cell and Optimize the $Ca^{2+}$Extrusion Pump

Adequate $Mg^{2+}$ is critical for both of these defense activities: a) it functions as a competitive, passive $Ca^{2+}$ channel "blocker"; b) it is the required cofactor for the ATPase-catalyzed, $Ca^{2+}$ extrusion pump; c) it is required as a cofactor for the mitochondrial enzymes involved in any exaggerated energy production mandated in the hair cell by intense sound. For these reasons adequate $Mg^{2+}$ is necessary for hair cell defense. Hypomagnesemia is associated with hearing loss from sound-induced hair cell trauma, but the latter can be reduced by supplementation with oral $Mg^{2+}$. Hypomagnesemia is commonly present in the aging and in diabetic populations and contributes to the fact that each of these groups is more prone to sound-induced hair cell and efferent synapse damage.

Although glutathione peroxidase (GSHPx) levels, necessary for the intracellular synthesis of GSH, appear naturally to rise with aging, reflecting a compensatory increase in the GSH needed to counter the rising levels of ROS associated with increasing age, intracellular GSH remains low in the presence of hypomagnesemia.

There is evidence that ginkgo biloba is also helpful in reducing acute sound induced hair cell trauma, perhaps relating to its vasodilatative effect. Given the importance of maintaining the normal state of vascular mid-dilatation and the sudden requirement for this at the time of noise trauma the effectiveness of ginkgo biloba is and therefore its inclusion in this patent is understandable.

#### 2. Improve the Multilayered, Coordinated, Intracellular Molecular Defenses Against ROS

ROS are produced by intense sound and contribute to hair cell trauma by a variety of etiologies including: a) immediately traumatic intracellular events, b) induced vasoconstriction and c) excitotoxic (glutamate) destruction of the terminal dendrites of primary auditory neurons postsynaptic to the sensory inner hair cells.

Intracellular defenses against the destructive effects of ROS require a number of components functioning as a team, including: 1) the GSH/GSHPx system which maintains production and optimizes its catalytic functions (e.g., cysteine, melatonin, riboflavin, selenium); 2) sulfhydryl contributors that reduce the ROS "load" on the GSH/GSHPx system (e.g., lipoate, taurine) and 3) molecules involved in ROS scavenging (e.g., D, alpha-tocopherol, ascorbate).

These ROS scavengers function as an interdependent, dynamic intracellular ensemble. Endogenous peptides like GSH and GSHPx play the predominant role in many regulatory processes, usually with high specificity and potency, and rapid degradation; the latter is necessary for flexible regulation.

Such a system requires a healthy means of rapid regeneration and maintenance of adequate levels of complementary components. E.g., it is well established that once D, alpha-tocopherol has functioned as a reductant of ROS—preventing lipoproteins from being oxidized, endothelial cells from becoming dysfunctional and DNA from being damaged—it exists in an oxidized form, tocopheroxyl. Ascorbate, in turn, acts as a reductant of tocopheroxyl and rejuvenates alpha tocopherol, but in the process is itself oxidized to a prooxidant, dehydroascorbate. Finally, GSHPx, activated by its cofactor selenium and utilizing reduced GSH as its substrate, completes the cycle and rejuvenates ascorbate. (These activities emphasize the often-overclocked fact that many antioxidants like ascorbate and alpha tocopherol can be driven into prooxidant states. It importantly defines how imperative it is to maintain a balanced, synergetic intracellular milieu and avoid artificially converting a cellular physiologic state into one that is pathologic.)

Lipoate (thioctic acid), another potent antioxidant, is also regenerated through redox cycling and raises intracellular GSH levels by providing thiols. Since GSH (a thiol-requiring antioxidant) cannot effectively be taken orally (see below) while alpha-lipoic acid can, the latter is effective as a dietary supplement in maintaining intracellular GSH levels.

GSH levels cannot be raised directly by supplemental administration in the diet because it is produced intracellularly from the amino acids glutamic acid, cysteine and glycine; cysteine is the functional component. As the functional unit of GSH, cysteine can be supplied effectively by providing a GSH prodrug, such as N-acetyl-cysteine (NAC), 2-oxothiazolidine-4-carboxylate (OTC) or mercaptopropionylglycine (MPG). In fact, the GSH prodrug OTC has been shown rapidly to restore GSH when the latter is acutely depleted.

$Zn^{2+}$ is a necessary trace element in GSH synthesis, as is $Mg^{2+}$.

GSH presence in the brain is enhanced by pineal melatonin via this neurohormone's ability to increase the mRNA of GSHPx.

In appropriate doses in sequence and in concert, these several components - D, alpha-tocopherol, ascorbate, lipoate, GSH, NAC, OTC, MCG, $Zn^{2+}$, selenium and melatonin—function efficiently to reduce the cell damaging effect of ROS while avoiding the cell damage that each can exert should they accumulate in their prooxidant form.

In summary:

An important etiology of hearing loss from acoustic over stimulation is the generation of ROS. Those ROS not removed by limited, resident antioxidant defenses cause significant damage to the sensory cells of the cochlea. Studies have shown that GSH inhibition increases the susceptibility of the cochlea to noise-induced damage and that replenishing GSH by the administration of the GSH prodrug OTC, presumably by enhancing the availability of cysteine (thiols), attenuates noise-induced cochlear damage.

Intracellular hair cell GSH is reduced when oxidant stress is increased to a level that depletes or disorganizes inherent, multilayered intracellular ROS defense systems. As a defense, GSH synthesis is markedly and selectively up regulated in the lateral wall by noise exposure (which imposes a higher requirement for the components required for synthesis). This up-regulation, presumably, is in response to the robust consumption of GSH as it is over-utilized in scavenging elevated, noise-induced, toxic levels of ROS.

There is a rise in GSHPx levels with aging; presumably merely to compensate for the increased amounts of GSH required to counter elevated, universal levels of ROS.

Unfortunately, GSH is often reduced because of hypomagnesemia, also common in the aged. These facts underline the particular importance of supplementing $Mg^{2+}$ in an aging population to maintain appropriate intracellular synergetics for resisting sound-induced hearing loss.

Alpha-tocopherol, ascorbate, selenium and $Mg^{2+}$ are commonly deficient even in average diets. These components, GSH prodrugs and lipoic acid must be supplemented orally to meet the ROS hair cell defense demands of intense or prolonged sound exposure and the depredations of aging. The same is true for melatonin, which may be progressively reduced during aging either because of increased intracellular ROS demands, inadequate pineal production or both.

## II. Chronic Labyrinthine Ischemia

Progressive sensorineural hearing loss (PSNHL) is often caused by chronic labyrinthine ischemia, either from by hematologic factors (blood viscosity and/or rigidity of the red blood cells) or non-hematologic factors (tissue perfusion pressure, blood vessel diameter).

### A. The Synergetics of blood Flow are Nonlinear

The nonlinear synergetics of blood rheology may shift rapidly causing abrupt reductions in flow and result in acute, localized loss of tissue perfusion and cell death. These may be associated with immediate hearing loss and cell damage. The latter may contribute continuously to PSNHL. This pathology is a pulsed, progressive, permanent and yet preventable disability.

That these phase shifts in blood rheology, with their associated changes in available oxygen and nutrients, can be sound induced and cause permanent hearing loss has been established. That they can be prevented is one basis for this patent.

The patterns inherent in the nonlinear dynamics of blood flow have valuable clinical implications for both the acute, traumatic hearing loss described above and for the chronic progressive hearing losses relating to chronic labyrinthine ischemia.

There is a strong and complex association between aspects of blood rheology and hearing impairment. These must be understood.

Two separate items are strongly associated with sensorineural hearing impairment: a) bulk Theological properties of blood and b) the Theological properties of individual red blood cells (RBCs, erythrocytes). Bulk flow abnormalities appear to be more important at lower frequencies, while defects in RBC deformability are more detrimental to higher frequency hearing.

### B. Erythrocytes and the Concept of Flow Synergetics

#### 1. Deformability

Erythrocytes are the simplest cells in the human body. Formed as nucleated cells in the bone marrow, erythrocytes lose this element before their release into general circulation. Once in the circulation, an RBC assumes the shape of a biconcave disk. These non-nucleated cells have a changeable (dynamic) fluidity in flowing blood.

Cellular deformability is influenced by three distinct cellular components: a) cell shape, which determines the ratio of cell surface to cell volume; b) cytoplasmic viscosity, which is regulated by intracellular hemoglobin concentration; c) membrane properties including shear, negative surface charge (sialic acid) and the coefficient of surface viscosity.

The biconcave disk shape of normal erythrocytes creates an advantageous surface-to-volume ratio that allows the erythrocyte to undergo marked deformation while maintaining a constant surface area. Concurrently the extensive surface-to-volume ratio enhances respiratory gas exchanges in the lungs and in the peripheral circulation. Maintenance of deformability is essential for these cells to negotiate successfully small passageways in the microcirculation. Erythrocytes, which have a diameter of 8 $\mu$m, can squeeze through interendothelial slits of 0.5 $\mu$m without rupture. This incredible, reversible deformability is permitted by the presence of a flexible cytoskeleton of interconnected filamentous proteins anchored to the inner part of the plasma membrane.

#### 2. Cell Membranes

Like other cellular membranes, that of the erythrocyte is a selectively permeable lipid barrier with specific ion pumps, channels and gates.

The deformability of the erythrocyte membrane is determined by lipid-protein interactions. There are three types of membrane lipids: phospholipids, cholesterol and glycolipids. The phospholipids form a membrane bilayer with hydrophilic groups oriented towards the exterior and hydrophobic hydrocarbons oriented towards the interior. About half of the membrane lipids are phospholipids, about half cholesterol lipids. The most important interacting membrane protein is glycophorin that makes up 75% of all erythrocyte membrane proteins.

#### 3. Fluid Dynamics

Any solid particle suspended in a shearing fluid is rotated by the flow characteristics imposed by viscous drag. Solid spheres are driven into continuous rotation while irregularly shaped particles (e.g., solid ellipsoids like rigid RBCs) are driven into irregular movements. Although a fluid droplet may be spherical at rest it is progressively deformed into a prolate ellipsoid as shear rates rise. Its major axis then becomes oriented more or less parallel to the direction of flow. In addition, the deformed fluid droplet acquires the rotational movement of the suspending fluid, which minimizes the consequences to it of gyrations encountered during shearing: a) droplet (cellular) aggregation, crowding and collisions are markedly reduced and b) entropy generation is minimized.

These phenomena variably occur in any sheared suspension of RBCs. When subjected to physiologically high shear stresses the highly flexible RBCs behave like liquid droplets and blood undergoes a phase transition into a highly fluid emulsion. At low rates of shear these same cells behave more like quasi-elastic, semi-solids. In summary: RBCs at normal, relatively high physiologic levels of shear behave much more like a fluid than a solid: hence the term Shear-Induced Fluidality (SIF) to designate their behavior.

SIF can be explained by several features of erythrocyte mechanics: a) these cells consist of a flaccid membranous bag incompletely filled with a concentrated, very fluid solution of cytosolic macromolecules, b) the cytoplasm convectively mixes oxyhemoglobin between the cytosol and the plasma, movement that markedly accelerates oxygen uptake and release, c) unique intra-cytosolic laminar flow is induced by rotational slippage of the RBC membrane around the cytosol (called "tank treading"); this membrane movement is driven by surrounding shear forces.

When blood is properly driven, SIF spontaneously enhances its fluidity so that the cellular elements (RBCs, white blood cells, (WBCs), et al) can easily negotiate small vessels despite the fact that resting capillaries are substantially smaller in diameter than they.

Physiological or pathological changes in the dynamics of blood flow associated with these processes responsively can induce sudden, non-linear changes of flow and fluidity.

- At rest (zero shear) human RBCs regularly combine into rouleaux and three-dimensional networks of rouleaux.
- At low shear rates between 0.01 and 10/s viscidation is increased by RBC aggregation.
- At high shear rates between 10 and 1000/s fluidity is improved by changes in RBC orientation and deformation (see above).

As one proceeds from stationary to rapidly moving blood flows, progressive increases in dispersion and deformation of the RBCs lead to changes in the appearance and the behavior of the blood: from a viscous suspension to an emulsion-like, self-lubricating fluid.

Phase jumps from slow movement associated with aggregation, to rapid movement associated with dispersion and deformation can occur—a circular causality, wherein causes and consequences are inseparably interwined. Although at normal physiological high flow rates these dynamics permit an efficient coordination of perfusion, the opposite will occur in slowly moving blood, which phase shifts into a self-viscidizing mode once the shear stresses fall below a critical level. The perfusion pattern has now become chaotic, entropic, and nonlinear.

In all segments of the normal macro and microvasculature, both the shear rates and the shear stresses are high, in the order of magnitude of 100-1000/s and vary inversely to the vessel diameter. The controlling parameter is the intravascular pressure: When the local vascular pressure drops sufficiently to reduce shear forces, the low-flow behavior of increasing cellular aggregation and increasing viscidation begin leading to strongly disordered flow patterns.

The above admittedly lengthy explanation helps explain why vasoconstriction (using this term in its broadest sense and developed more extensively below) caused by either intense/prolonged sound or chronic labyrinthine ischemia diminishes the physiological defenses necessary for the preservation of blood fluidization and greatly increases the probability of a local, auditory, nonlinear phase jump toward chaotic, cellular aggregation. The result is otherwise preventable auditory tissue damage. The prevention of the latter is the focus of this invention.

## C. Vasoconstriction

### 1. Microvascular Regulation

Disturbed microvascular regulation and its resulting vasoconstriction create disastrous phase shifts in blood flow that are integral to auditory damage and hearing loss.

A balanced biochemical relationship between nitric oxide (NO) and ET-1 mediates local blood flow and many other facets of systemic vascular tone.

NO is a highly soluble gas formed within endothelial cells by the action of the constitutive enzyme nitric oxide synthetase (eNOS). NO activates guanylate cyclase and increases guanosine monophosphate (cGMP) within the vascular musculature. In turn, cGMP produces relaxation and dilatation of the vessel. NO is the most powerful initiator of vasodilation known, except for histamine. Furthermore, its continuous constitutive production maintains the normal vascular system in a physiologic state of partial vasodilation. Importantly, in an aging population increasingly affected by hearing loss and in whom atherosclerosis is universal, the ability of the vascular endothelium to produce NO is lessened because of reduced local levels of eNOS.

ET-1 is also formed within and secreted by endothelial cells. ET-1 reacts with local receptors on smooth muscle cells to produce a powerful and long-lasting vasoconstriction. Aged or unhealthy endothelial cells particularly release ET-1, e.g., in the presence of atherosclerosis or in the presence of locally bound aggregates of endothelial leukocytes or platelets. The smooth muscle contraction produced by ET-1 strongly opposes the vascular smooth muscle cell (VSMC) relaxation of NO. This causes spotty or widespread vasoconstriction of the small vessels of the cochlea with resulting local hypoxia and hair cell atrophy.

This critical balance between constitutive NO and ET-1 mediates the regulation of blood flow within the auditory microvasculature.

### 2. Vascular Disease

Localized vascular disease can exist in a variety of forms and result in a variety of pathological clinical conditions including chronic labyrinthine ischemia, which eventually result in hearing loss. All are associated with a reduction of oxygen delivery to surrounding, dependent tissues. In the ear there are two tissues particularly vulnerable to hypoxia:

a. The hair cells of the cochlea.

b. The afferent dendrites of the VIIIth nerve. These dendrites are vulnerable to damaging excitotoxicities that result from the excessive release of glutamate at the synapse after intense/prolonged sound. (Hypoxia and ROS reduce available GSH, which is necessary to maintain glutamate below exorhitant excitotoxic levels. (See discussion above)

A reduction in cochlear oxygen delivery may follow acute or chronic, segmental or widespread vascular spasm or prolonged vasoconstriction secondary to a physical or functional reduction in the vascular lumen. This luminal reduction may be caused by or be associated with hypertrophy of the vascular muscle wall (the media), the accumulation of atherosclerotic plaque, platelet agglutination, RBC rigidity, disturbed laminar flow, blood fluidity or local inflammatory swelling and leukocytic accumulation. Any and all of these often occur with aging or in association with other systemic disease: diabetes, hypertension, dyslipogenesis, hyperinsulinemia, arteriosclerosis, thyroid disease, etc. Although vascular insufficiency at specific tissue sites is widely variable and not predictable with certainty, the fact that most patients with hearing loss are over 45 years old makes the frequency of these risk factors and the frequency of vascular insufficiency, high in this clinical group.

Any proposed therapy should attempt to reduce the negative influences of the above general risk factors and reduce local cochlear vascular insufficiency, in addition to reducing noise exposure. For example: A therapeutic reduction of those endothelial abnormalities which contribute to (or are created by) risk factors which compromise local vascular integrity, will reduce the potential for cochlear hair cell failure where microvascular dysregulation or vasoconstriction is significant. If a reduction of vascular risk factors is united with a reduced exposure to noise, the combined effects of a well-oxygenated hair cell and lessened excitotoxicity of VIIIth nerve dendrites will reduce progressive hearing loss.

D. Insulin Resistance and Diabetes Mellitus

Tobacco smoking, obesity, high fat diets and increasing age are all associated with elevations of tumor necrosis factor alpha (TNF-α) and an increased incidence of diabetes mellitus type 2 (NIDDM). TNF-α elevations and NIDDM are both closely associated with hyperinsulinemia and reduced insulin sensitivity. Hyperinsulinemia and reduced insulin sensitivity, which may exist in 25% of an otherwise apparently healthy general population, are associated with disturbed vascular laminar flow, endothelial dysfunction, dyslipogenesis and hypertension—in brief, with vascular insufficiency, vasoconstriction and reduced deformability of RBCs. The hearing loss and tinnitus population is predominantly represented by an older age group and which is not immune to the existence of smoking, obesity, NIDDM, background levels of hypertension, etc. The epidemiological relationships that exist between diabetes, aging and neurosensory hearing loss has been discussed above.

SUMMARY OF THE INVENTION

The present invention resides in pharmaceutical preparations for use as oral dosage forms or transmembrane delivery forms. The preparations contain specific therapeutic biofactors and biomolecules selected because of their particular and critical physiological effects. These are combined in highly defined groups and amounts to achieve maximum complementarity of action.

This invention prevents hair cell damage, VIIIth nerve dendritic damage, labyrinthine ischemia and associated hearing loss by improving local cochlear health and local and systemic vascular endothelial health. This results from the advantageous modulation of intracellular $Ca^{2+}$ waves, the maintenance of vascular intraluminal fluidity by increasing cellular levels of NO (thereby augmenting the beneficial effects achieved by maintaining physiologic levels of vascular cGMP), by maintaining RBC deformability and by reducing the vascular risks associated with hyperinsulinemia secondary to reduced insulin sensitivity.

Because it maintains physiologic levels of NO and cGMP the invention improves general and local blood flow, increases reparative vascular endothelial cell proliferation, enhances inherent antithrombotic activities, reduces endothelial permeability, inhibits VSMC proliferation and inhibits cellular (neuronal and glial) apoptosis.

The systemic diseases most commonly associated with PSNHL are diabetes mellitus and hypertension—this is especially true when they coexist, as they often do. To the extent hyperinsulinemia and reduced insulin sensitivity exist in a significant portion of the general population, they also exist in the hearing loss population and are risk factors for hearing loss, notably although not exclusively, from changes in RBC rheology. By improving insulin sensitivity via (inter alia) insulin mimicry and voiding possibilities of hypomagnesemia, and concurrently reducing or preventing the clinical complications of hyperinsulinemia, the invention will reduce the vascular pathologics coexistent with labyrinthine ischemia and will aid in maintaining normal RBC deformability. In consequence, the invention will reduce the risks of sensorineural hearing loss associated with diabetes and hyperinsulinemia.

COMPONENTS OF THE INVENTION

Arginine

Dietary L-arginine improves NO-dependent vasodilatation and reduces vascular oxidative stress. L-Arginine exerts antihypertensive and antiproliferative effects on vascular smooth muscles, restores NO production and reduces the vascular release of superoxide anions. Endothelial dysfunction can be improved in both the coronary microvasculature and in epicardial coronary arteries by the administration of L-Arginine. Because an L-arginine-deficient diet reduces the hearing of treated animals a similar effect can be expected in the labyrinth vasculature. Indirectly inhibiting the production of NO by inhibiting eNOS increases the vulnerability of the myocardium to ischemia. Restoration of NO activity induces regression of preexisting intimal lesions providing evidence that long term L-arginine therapy can be clinically beneficial in lessening atherogenesis.

Absorption: Absorption of L-arginine is highest in the upper three gastrointestinal regions and least in the ileum. But no preferential site of absorption has been found.

Pharmacokinetics: The gastrointestinal uptake of dietary arginine when the stomach is in the "fed" state is about 20% to 38%.

Ascorbate

The outer cochlear hair cells transform sound into electrical signals, beginning the neural auditive process. In animal experiments antioxidants, including vitamin C, protective the outer hair cells of the cochlea.

The phase transfer rejuvenation of alpha tocopherol by ascorbate must occur to maintain and amplify D, alpha-tocopherol's chain-breaking effect on lipid peroxidation, the ultimate protection from free radical damage to cell membranes. (Synergism with alpha tocopherol is shared by two other components of this patent, ubiquinone and quercetin.) Ascorbate is also synergistic with taurine for $HOCl^-$ defense, with GSH or selenium for hydrogen peroxide defense and with SOD, zinc or copper for superoxide defense. (Superoxide and excess NO form peroxynitrite, an important tissue-damaging reactive species; GSH and ascorbate protect efficiently in this area, perhaps because ascorbate mimics stimulation of SOD activity by GSH.)

Ascorbate improves impaired acetylcholine-induced vasodilation by preventing oxygen free radical endothelial dysfunction and the associated reduction of constitutive NO.

Ascorbate is retained on the exterior cell surface of human erythrocytes, where it helps to protect the membrane from oxidant damage originating outside the cells. The ascorbate protects D, alpha-tocopherol in the erythrocyte cell membrane by a direct recycling mechanism.

Working together, ascorbate and D, alpha-tocopherol, maintain erythrocyte membrane integrity and deformability and preserve the antioxidant reserve of whole blood.

Absorption: Natural and synthetic ascorbates are avidly absorbed in the first 30 cm of jejunum.

Pharmacokinetics: As the daily oral dose vitamin C is increased, the concentration of ascorbic acid in the plasma and other body fluids does not increase proportionally, but approaches an upper limit. Analysis indicates that both saturable gastrointestinal absorption and nonlinear renal clearance act additively to produce a ceiling effect in plasma concentrations. As a consequence, there is no pharmacokinetic justification for the use of extremely large doses of vitamin C. Supplemental doses of ascorbate must be chosen carefully to avoid unwanted side effects. For example, recurrent renal stone formers or patients with renal failure who have a defect in vitamin C metabolism or patients with oxalate metabolism should restrict daily vitamin C intakes to approximately 100 mg.

### Chromium

Chromium (Cr) reduces insulin resistance. Insulin resistance precedes almost all diabetes mellitus type 2, which accounts for 95% of all diabetes mellitus.

Hearing loss has long been associated with diabetes mellitus. Of all the metabolic, hormonal and vascular disorders considered to cause $PSNH_L$, diabetes is the disease most commonly described. A hearing aid is three to four times more prevalent in patients with diabetes mellitus type 2 than in subjects without diabetes of the same age; not surprising, since about 50% of type 2 diabetic patients demonstrate impaired hearing.

Although Cr is an essential nutrient required for sugar and fat metabolism, routine dietary intake of Cr for humans is suboptimal. It has been proposed that 90% of American's diets are deficient in this essential trace element. Most diets contain less than 60% of the minimum suggested daily intake of 50 micrograms. Insufficient dietary intake of Cr leads to signs and symptoms that are similar to those observed for diabetes. Supplemental Cr given to people with impaired glucose tolerance or with overt diabetes improves blood glucose, insulin, and lipid variables. Any response to Cr, however, is dependent upon the form and amount of supplemental Cr. For example, trivalent Cr ($Cr^{+3}$) has a very large safety range and there have been no documented signs of toxicity in any of the $Cr^{+3}$ nutritional studies up to levels of 1 mg per day.

Absorption: Even with significant dietary Cr intakes, only a small fraction of the ingested Cr, is absorbed; most, congruent with intake, is excreted in the stool. Urinary Cr is constant from day to day. The Cr balances (apparent net retention) remain in positive equilibrium. In one small study, the average apparent net absorption of Cr was 1.8%.

Pharmacokinetics: Principal Cr concentrations are found in the liver, spleen, soft tissue, and bone. Most dietary nutrients and metabolites do not alter Cr retention or distribution. The regulation of Cr homeostasis appears to be modulated by excretion.

### CoQ10

Coenzyme Q10 (CoQ10) has already favorably been evaluated in the clinical treatment of heart disease. In the otolaryngological field, it has been reported that CoQ 10 is effective in promoting recovery from acute, sudden deafness. The pharmacokinetics of CoQ10 in the inner ear indicate that CoQ10 is effective in promoting recovery of damaged auditory hair cells by preventing respiratory metabolic impairment of these cells due to hypoxia. In CoQ10 treated animals, the chronic depression of hearing is milder than that in the control animals.

In addition to assisting ascorbate in the phase transfer rejuvenation of D, alpha-tocopherol, CoQ10 further complements alpha tocopherol by directly inhibiting lipid peroxidation. As one example: low-density lipid 3 (LDL3)-bearing serum, the densest of the three LDL subfractions, shows statistically significant lower levels of CoQ10. This condition is associated with elevated hydroperoxide levels when compared with the lighter counterparts. After CoQ10 supplementation, LDL3 responds with a significant decrease in the hydroperoxide level. These results support an hypothesis that raising CoQ 10 endowment in subfractions of LDL lessens their oxidizability.

Absorption: Supplemental oil-based capsules of CoQ10 elevate CoQ10 in plasma by 178% while granular preparations increase COQ10 in plasma by 168%. Each form is therefore an acceptable delivery vehicle.

Pharmacokinetics: After oral administration of 100 mg of d5-CoQ10 to sixteen healthy male subjects, the mean plasma CoQ10 level attained a peak of 1.004+/−0.37 micrograms/ml within 6.5 to 8 hours after administration and the terminal elimination half-life was 33 to 38 hours. In most of the subjects, plasma d5-CoQ10 showed a second peak 24 h after dosing. This unusual plasma level curve can be explained by a 'compartment' model in which absorbed CoQ 10 is taken up by the liver, transferred mainly to very low-density lipids (VLDL) and distributed to the systemic blood.

### Folate

Age-related auditory dysfunction may be associated with poor vitamin B- 12 and folate status.

In 1992–93, an epidemic outbreak of peripheral neuropathy (50,862 cases; (incidence rate: 461.4 per 100,000) affected Cuba. Clinical forms included retrobulbar optic neuropathy, sensory and dysautonomic peripheral neuropathy, dorsolateral myeloneuropathy, sensorineural deafness, dysphonia and dysphagia, spastic paraparesis, and mixed forms. Deafness produced selective high frequency (4–8 kHz) hearing loss. Intensive searches for neurotoxic agents—in particular organophosphorus esters, chronic cyanide and trichloroethylene intoxication—were negative. However, treatment of patients with B-group vitamins and folate produced rewarding results. Supplementation of multivitamins to the entire Cuban population curbed the epidemic. Overt malnutrition was not present but a deficit of micronutrients, in particular thiamine, cobalamine, folate and sulfur amino acids appears to have been a primary determinant of this epidemic.

Homocysteine is an independent risk factor for cardiovascular disease. Hyperhomocysteinemia is associated with arteriosclerosis, atherosclerosis, decreased GSHPx activity, vasoconstriction, endothelial toxicity and thromboembolic events and the prevalence of arterial occlusive disease is high mild in young patients with hyperhomocysteinemia. In about 90% of such patients, treatment with vitamin B6 plus folic acid normalizes the homocysteine concentration. Reducing homocysteine-induced endothelial dysfunction complements the anti-vasoconstrictive and anti-thrombogenic components of this invention. The three key biofactors that favorably alter homocysteine metabolism are included in the invention: folic acid, pyridoxine and cyanocobalamin ($B_{12}$).

Folic acid stimulates BH4 regeneration. This is an essential cofactor required for the conversion of L-arginine to NO under the influence of Type III NOS (constitutive endothelial eNOS) within the endothelial cell membrane (see details above). Additionally, folic acid reduces the catabolism of NO and improves the bioavailability of endothelial-derived NO.

Absorption: Folic acid is absorbed in the first 30 cm of the jejunum by both saturable and diffusional routes.

Pharmacokinetics: Folic acid is a coenzyme which humans, unlike bacteria, cannot synthesize de novo; therefore it is a dietary essential. Folic acid is converted to the active coenzyme tetrahydrofolate (THF) by repeated hydrogenation of the pterin ring. The coenzyme THF is then capable of one-carbon-residues transfers of different oxidation states.

### Gingko Biloba

The main mechanisms of action of Ginkgo biloba are vasoregulation (increased blood flow), platelet activating

factor antagonism and prevention of membrane damage caused by free radicals, all activities that reduce tissue ischemia. Since ischemia is one pathogenic mechanism behind acute cochlear deafness, it is understandable that Ginkgo biloba significantly improves recovery from acute cochlear deafness and that used in animals it reduces sound damage from white noise or from a pure tone at 4.5 kHz.

Hearing loss secondary to hematologic factors may be lessened by Ginkgo biloba supplementation. In a prospective study, twenty patients with a long history of elevated fibrinogen levels and plasma viscosity were treated with the Ginkgo biloba extract, EGb 761 (240 mg tablets a day for a period of 12 weeks). Fibrinogen levels and hemorrheological properties significantly improved.

Controlled clinical trials have been evaluated in a meta-analysis to evaluate the effectiveness of Ginkgo biloba on symptoms of cerebrovascular insufficiency in old age. All the included studies were placebo-controlled, randomized, double blind studies using a daily dosage of 150 mg ginkgo biloba. Seven of eight studies confirmed the effectiveness of Ginkgo biloba compared to a placebo, while only one was inconclusive.

Absorption: The absorption of EGB is about 60%. Different formulations of Ginkgo biloba extracts (e.g., capsules, drops or tablets) appear to be bioequivalent.

Pharmacokinetics: The ginkgolides and bilobalides, which are compounds extracted from the dried leaves of the Ginkgo biloba tree, have high bioavailability when given orally during fasting. The bioavailability coefficients (FAUC) have mean values equal to 0.80 (+/−0.09), 0.88(+/−0.21) and 0.79(+/−0.3) for Ginkgolide A, Ginkgolide B and Bilobalide respectively. Food intake does not change FAUC quantitatively but increases Tmax.

## Glutathione and Glutathione Prodrugs

A principal mechanism of hearing loss due to acoustic over stimulation is reactive ROS generation and ROS not removed by antioxidant defenses cause significant damage to the sensory cells of the cochlea. GSH inhibition increases the susceptibility of the cochlea to noise-induced damage. Replenishing GSH, by enhancing availability of cysteine, attenuates noise-induced cochlear damage. As an example, the GSH prodrug OTC promotes rapid restoration of GSH when GSH is acutely depleted. GSH synthesis is markedly upregulated selectively in the lateral cochlear wall by noise exposure in response to the consumption of GSH as the latter is utilized in scavenging ROS. This emphasizes the importance of adequate supply of GSH and supports supplementation of GSH prodrugs for the prevention and treatment of noise-induced hearing loss (NIHL). Depletion of endogenous GSH potentiates $NIHL_1$ whereas replenishment of GSH attenuates $NIHL_2$.

The body possesses complex protective antioxidant systems against ROS production, such as dismutase superoxides, catalases, metallic ion sequestration, enzymes which degrade proteins damaged by ROS, metabolizing hydroperoxides, DNA repair processes, vitamins E, C and, in particular, the GSH enzyme system. A physiological steady state is established under normal conditions between the production of oxidants and their neutralization by antioxidants.

GSH levels cannot be raised predictably by supplemental administration in the diet. Because of peptidase activity in the small intestine most peptides undergo rapid degradation in the lumen of the gastrointestinal tract. There is evidence that limited uptake of small (dipeptides and tripeptides) is possible. But the bioavailability of orally administered peptides generally is low. Aside from this, even if absorption from the gastrointestinal tract is sufficient to raise plasma levels, questions remain regarding their ability to gain passage into cells.

Concern that raising plasma levels of GSH could stimulate a negative feedback loop down regulating intracellular GSH production should be addressed. Therefore, until these issues are resolved it is probably best to administer GSH precursors.

Reduced GSH is important and ubiquitous. It is necessary for intracellular transduction signaling, for the modulation of cellular apoptosis and necrosis, and the modulation of red blood cell fragility. During its function as an antioxidant it is oxidized to disulfide glutathione (GSSG). This oxidation importantly protects vascular endothelium from free radical damage. GSH inhibits the peroxidation of LDL directly reducing atherosclerotic and vasoconstrictive risks, and oxLDL-induced mitochondrial DNA mutations. Besides their influence upon atherogenesis and vasoconstriction, these effects are linked to a variety of specific sensory neuropathies.

GSH plays multiple roles in the nervous system including free radical scavenging, redox modulation of inotropic receptor activity and neurotransmission. GSH depletion enhances oxidative stress and increases the level of neuroexcitotoxic molecules; in distinct neuronal populations including the auditory system, both of these events can initiate cell death.

Exposure to glutamate, an important neurotransmitter, causes depletion of intracellular mitochondrial GSH leading to the accumulation of ROS and, ultimately, apoptotic cell death. Cells which have enhanced rates of GSH regeneration due to higher activities of the GSH metabolic enzymes gamma-glutamyl cysteine synthetase and GSH reductase appear to be resistant to glutamate-induced ROS. Not surprisingly, maintenance of intercellular GSH level appears to exert a neuroprotective effect.

Because the protection of the electron acceptor homocysteine thiolactone declines with aging, homocysteine levels frequently increase. GSH levels are lowered by homocysteine.

GSH is low in the presence of hypomagnesemia. Hypomagnesemia is commonly present in the aging (and the diabetic) population.

Redox-sensitive mechanisms are involved in VSMC growth with attendant ischemia and apoptosis. ROS that promote VSMC growth are inhibited by GSH. This might be expected since upon oxidation micronutrients need to be regenerated in the biological setting, hence their need for coupling to nonradical reducing systems such as GSH/GSSH or NADPH/NADP+ and NADH/NAD+.

Cysteine is a necessary thiol precursor of GSH. GSH predecessors (NAC, 2-OTC, MPG) supply cysteine residues to cells for GSH synthesis. These prodrugs, which do in fact increase GSH levels, protect endothelial cells from atherosclerotic damage, perturbations of laminar flow, VSMC hypertrophy, cell detachment, etc., help to preserve a normal NO/ET-1 ratio and protect against $NIHL_1$.

Absorption: NAC is one example of a precursor of GSH used in this invention. Intestinal absorption of NAC is satisfactory. After an oral dose of 200 to 400 mg of NAC, peak plasma concentration is achieved within 1 to 2 hours. The upper jejunum is a principal site of some, but very limited, GSH absorption. This low GSH

bioavailability is not increased by higher doses. Orally administered GSH at reasonable levels does not affect the circulating concentrations of GSH, whereas NAC administration increases the GSH content in lungs, blood and/or liver.

Pharmacokinetics: The administration of NAC increases hepatic cysteine placing it on a path for the modulation of systemic GSH levels. Following supplementation, pharmacokinetic and pharmacodynamic studies of NAC demonstrate elevated GSH levels in plasma, RBC and peripheral blood lymphocytes (PBL), elevated cysteine levels in plasma and increases in two, GSH-metabolizing enzymes, glutathione S-transferse and oxidized glutathione reductase, in the PBLs. These studies have established NAC as the precursor of GSH.

alpha-Lipoic Acid

Dose-dependent otoprotection in animals is conferred by lipoate by sparing of the cochlear antioxidant defense system. The proglutathione metabolic antioxidant alpha-lipoic acid (LA) (1, 2-dithiolane-3-pentanoic acid) is a low molecular weight substance that is absorbed from the diet, is both water- and lipid-soluble and crosses the blood-brain barrier. Within cells and tissues the salt form (alpha-lipoate) is reduced to an even more active structure, dihydrolipoate, which is exported to the extracellular medium; hence, antioxidant protection is afforded to both the oxidized and reduced forms, within both intracellular and extracellular environments. LA acts as a mitochondrial coenzyme that is involved in reversing declines in cellular $O_2$ consumption and impaired mitochondrial membrane potentials. It is important in decreasing malondialdehyde (MDA) levels (an indicator of lipid peroxidation), in regenerating ascorbic acid and increasing GSH levels in rats.

Since alpha-lipoate and dihydrolipoate forms have been shown to be potent antioxidants, both the oxidized and reduced forms of LA have antioxidant activity and either form can regenerate through redox cycling other antioxidants like ascorbic acid and alpha-tocopherol, and raise intracellular GSH levels. LA can be directly administered as a dietary supplement, whereas GSH cannot. These various features make it evident that LA may be useful in reducing the twin conditions of vasoconstriction and ischemia associated with acute or chronic cochlear damage.

After oxidative stress induced by hypoxia/reoxygenation and treatment with LA, there is distinct improvement of mitochondrial structure/function. Loss of GSH accompanied by concurrent mitochondrial dysfunction, can be inferred in vitro by losses of Complex I activity in male mouse brain slices and in vivo in selected regions of mouse CNS exposed to excitatory amino acids. The inhibition of Complex I is abolished by the maintenance of protein thiol homeostasis with pretreatment with GSH or with LA.

Transcription factor NF-kappaB is a cell-signaling pathway. It leads, for example, to gene expression in keratinocytes after exposure to solar UV radiation (UVR). Exogenous supplementation of antioxidants prevents UVR-induced photo-oxidative damage. While high concentrations of NAC can inhibit NF-kappaB activation, low concentrations of LA have a similar significant effect. These results indicate that the very efficient antioxidant properties of LA may lay in their selective action on NF-kappaB activation.

Reduced GSH is a cofactor for the glyoxalase system, a metabolic pathway that catalyses the detoxification of alpha-oxoaldehydes (RCOCHO) to corresponding aldonic acids ($RCH(OH)CO_2H$). The glyoxalase system protects cells from alpha-oxoaldehyde mediated formation of advanced glycation end products (AGE). AGE's are implicated in a wide variety of diabetic vascular abnormalities and, perhaps, in the pathogenesis of age related processes such as hearing loss and macular degeneration. Studies have found that incubation of cultured bovine aortic endothelial cells (BAECs) with AGE albumin results in decreases of GSH and ascorbic acid levels. This increased cellular oxidative stress leads to the activation of NF-kappaB and promotes the upregulation of various NF-kappaB-controlled genes, including endothelial tissue factor. However, the addition of LA before AGE exposure completely prevents depletion of GSH and ascorbic acid by inhibiting the release and translocation of NF- kappaB from the endothelial cytoplasm into the nucleus. Because LA reduces this AGE-induced NF-kappaB mediated transcription, the expression of endothelial genes such as tissue factor and the vasoconstrictor ET-1 is reduced.

Redox-sensitive mechanisms are involved in VSMC growth. ROS that promote VSMC growth are inhibited by GSH and also are negatively influenced by LA. In addition to lessening VSMC hypertrophy, supplemental thiols such as LA that increase GSH levels, protect endothelial cells from damage. This helps preserve a normal NO/ET-1 ratio and limit perturbations of laminar flow, thereby reducing the probability of the phase shift in blood flow associated with NIHL. By providing additional improvement in GSH synthesis and thus augmenting intracellular GSH, LA also improves NO-dependent, flow-mediated dilation.

Absorption: Non-saturable kinetics of LA in healthy volunteers is demonstrable from single oral doses in the range of 200 to 600 mg. Thioctic acid is a rapidly absorbed, racemate of R-(+)- and S-(−)-enantiomers of LA which acts as a powerful lipophilic, free radical scavenger. Oral LA supplementation in rats increases levels of free LA in the gastrocnemius muscle and increases total GSH levels in the liver and blood.

Pharmacokinetics: In one study, the absolute bioavailability of LA in humans after a 200 mg oral dose was 29.14+/−10.3%. In rats given oral doses of ($C_{14}$) LA, the area of ($C_{14}$) LA in the plasma concentration-time curve (FAUC) was 66% of that following similar intravenous administration.

Magnesium

In animal experiments, correlations were observed between serum $Mg^{2+}$ levels and noise-induced permanent hearing threshold shifts (NIPTS); dietary supplementation with $Mg^{2+}$ has been shown to reduce hearing loss in noise-exposed rats. Encouraged by this, the prophylactic effect of $Mg^{2+}$ in humans exposed to noise was investigated. The subjects were 300 young, healthy, and normal-hearing recruits who underwent 2 months of basic military training. This training necessarily included repeated exposures to high levels of impulse noises while using earplugs. During this placebo-controlled, double blind study, each subject received daily 167 mg of elemental $Mg^{2+}$ or placebo. NIPTS were significantly more frequent and more severe in the placebo group than in the $Mg^{+}$ group. NIPTS were negatively correlated to the $Mg^{2+}$ content of blood red cells and mononuclear cells. This study demonstrated the effectiveness of oral $Mg^{2+}$ supplementation in reducing hearing damage from conditions of high level, impulse noise exposure.

Cell membrane permeability is increased in hypomagnesemia, causing $Na^{+}$ and $Ca^{2+}$ influx and subse-

quent increased demands in energy-dependent, ion pumping. At the same time energy depletion in the hair cells appears to be one cause of noise-induced hearing loss, this energy exhaustion may be worsened by hypomagnesemia-induced vasoconstriction.

As discussed earlier, $Ca^{2+}$ in cochlear hair cells controls mechanical transduction, triggers neurotransmitter release and mediates efferent synaptic signaling. These activities are modulated by a $Mg^{2+}$-dependent outward current governed by the activity of plasma membrane Ca*-ATPase (PMCA). As a result the concentration of intracellular $Ca^{2+}$ is reduced and noise signaling is moderated.

The concentration of free $Mg^{2+}$ is important in the type of vascular disease that is associated with the hearing loss of aging. Effects on smooth muscle tone, serum lipid and lipoprotein levels, free radical production and energy metabolism are all linked to $Mg^{2+}$ concentration. Until recently it was not realized that $Mg^{2+}$ played such an important role in vascular dynamics. This lack of understanding, in part, reflects a poor appreciation of the progressive shortfalls in dietary $Mg^{2+}$ intake since the early 1900s and a failure to recognize that patients whose diets are deficient in this element are not necessarily hypomagnesemic. At the turn of the century average dietary intake of $Mg^{2+}$ in the USA was about 450 to 485 mg per day, which is accepted as a reasonable daily consumption to meet the requirements of cellular metabolism. The most recent figures indicate that a typical daily intake is now about one-half of this, so that there is now a typical dietary magnesium shortfall of 90 to 180 mg per day in this country.

The level of $Mg^{2+}$ is an important determinant of vascular tone, contractility and reactivity. By a variety of mechanisms, $Mg^{+2}$ functions both intracellularly and extracellularly to optimize the cytoplasmic free $Ca^{+2}$ level. Although the latter element is a critically important intracellular messenger, excessive cytoplasmic free $Ca^{+2}$ causes an increase in ET-1, with an associated decrease in blood flow, platelet aggregation and cell apoptosis. The correction of a $Mg^{+2}$ deficiency prevents or reverses the hypertensive, thrombotic and atherosclerotic effects of overabundant intracellular $Ca^{+2}$. $Mg^{+2}$ accomplishes this without interfering with normal $Ca^{+2}$ intracellular signaling. Clinically prescribed pharmaceutical calcium channel blockers (e.g., nifedipine) have amplitude-driven effects on $Ca^{+2}$ cellular signaling, which can be deleterious and occasionally fatal. Physiologic modulation of cell signaling requires a fine balance involving $Ca^{+2}$ flow patterns between the cell membrane, the plasma and the endoplasmic reticular substances, and $Ca^{+2}$ flow patterns within and from the cytoplasm. In addition to facilitating the ATPase energy requirements for modulation of $Ca^{+2}$ signaling, $Mg^{+2}$ exerts important regulatory effects on the precise subcellular location and concentration of both $Ca^{+2}$ and $Mg^{+2}$

Absorption: $Mg^{2+}$ is absorbed by active transport in the ileum although there is limited passive diffusion throughout the intestine.

Pharmacokinetics: There is a maximum intestinal bulk absorption of $Mg^{2+}$ of 8 mEq per meal with a curvilinear falloff and $Mg^{+2}$ absorption is negatively influenced by dietary protein: e.g., soybean protein, when compared with casein, decreases $Mg^{+2}$ absorption through its phytate component. Both this bulk absorption ceiling and the dietary protein influences speak to the importance of supplementing $Mg^{+2}$ in multiple doses per day.

## Melatonin

Melatonin, which is normally present in cochlear cells, has been shown to have a protective role on the postmortem activity of the outer cochlear hair cells of the rat, prolonging hair cell activity after death up to 7 times compared to untreated animals.

Melatonin, N-acetyl-5-methoxytryptamine, is a hormonal product of the pineal gland which is highly lipophilic and readily enters all cells and tissues in the body, including the brain. In addition to stimulating mRNA for both GSHPx and superoxide dismutase it is, itself, a very potent hydroxyl and peroxyl radical scavenger. It is suggested that as an intracellular free radical scavenger it is at least equal to GSH and vitamin E, affording protection to molecules (especially DNA) from oxidative damage. Melatonin's extremely high diffusibility is important for its scavenging action because this feature allows it to enter all cells and every subcellular compartment, including the nucleus. Melatonin is one of the premier molecules to protect the organism from oxidative damage.

Within 30 minutes exogenously administered melatonin causes a 2-fold rise in GSHPx activity in the brain. Brain GSHPx activity is higher at night than during the day and is correlated with high nighttime tissue melatonin levels. GSHPx is thought to be the principal enzyme for eliminating peroxides in the brain. It reduces the formation of hydroxyl radicals (formed via iron-catalyzed, Fenton-type reactions from hydrogen peroxide) by reducing this oxidant to water. Since the hydroxyl radical is the most noxious oxygen radical known, induction of brain GSHPx may be an important mechanism by which melatonin exerts its potent neuroprotective effects. In addition to increasing levels for GSHPx melatonin increases mRNA for superoxide dismutase, another important cellular antioxidant enzyme. These mRNA stimulatory effects are observed after both acute and chronic melatonin treatment.

Absorption: Ingestion of 3 mg melatonin causes a marked increase in serum melatonin (3561+/−1201 pG/mL) within 20 min. Although this is followed by a gradual decrease, the level still remains higher than the basal level at 240 min after ingestion.

Pharmacokinetics: When huge doses of melatonin (80 mg) are administered orally, changes in serum melatonin levels are best described by a biexponential equation with an absorption constant (ka) of 1.72 h-1 (half-life=0.40 h) and an elimination constant kel) of 0.87 h-1 (half-life=0.80 h). Peak serum melatonin occurs 60–150 min after its administration, remaining stable for approximately 1.5 hours.

## Nicotinamide

Improvements of rheological properties of blood and red cell deformability by alpha tocopherol nicotinate (TN) occur and are thought to be due mainly to reduced lipid peroxidation stress on the membrane of red blood cells. Whether the effect might also relate to the availability of nicotinamide (NAD) and nicotinate as an NAD precursor is reasonable, but has not yet been investigated. Alpha-tocopherol nicotinate 300 mg tid, after meals, for 3 months in Type 2 diabetes mellitus resulted in significant reductions of blood viscosity at different shear rates (e.g. −2.23 +/−2.82 p0.015, gamma=1.5 s(−1)) and viscoelasticity (p0.004); resistance of erythrocyte deformation (p0.001) and lipid peroxidation stress in red cell membrane (malondialdehyde or MDA reduced by 0.17+/−0.13 nmol l(−1) p0.005).

The water-soluble vitamin NAD, or niacin, has established itself as a useful oral supplement for patients who require reduction of plasma oxLDL, a decrease in plasma fibrinogen levels and stimulation of fibrinolysis. Such

supplementation decreases plasma fibrinogen and low-density lipoprotein cholesterol in subjects with peripheral vascular disease and can cause a significant elevation in liver NAD+, serving to ensure the continuous NADPH production via the pentose pathway which is important in maintaining protective levels of GSH. Additionally, recent findings suggest that the NAD+ precursors nicotinic acid and nicotinamide protect against oxidative stress and DNA damage by up-regulating the stress response genes GAPDH and G6PD.

The inhibitory effect of TN upon hydrogen peroxide-induced platelet aggregation has been found to be greater than that of either vitamin E alone or the simultaneous use of vitamin E and nicotinic acid. Nicotinic acid alone showed no inhibitory effect. It is suggested that the effect of TN is not due to any additive effects of vitamin E and nicotinic acid produced by hydrolysis, but to the unique and distinctive property of this molecule itself. Because of this unique property, TN is the molecular form of nicotinic acid most commonly used in products relating to this patent. The alpha tocopherol moiety of TN is taken up by red blood cell membranes and the nicotinate moiety is distributed among red blood cell contents. The main metabolite in both red blood cell contents and liver after a single orally administered dose of TN is nicotinamide.

Absorption: Immediate release dosage forms of nicotinamide achieve higher plasma levels than sustained release. Formulations at high doses produce nonlinear kinetics, e.g., a 10-fold increase in the dose of standard nicotinamide produces a 62-fold increase in the AUC.

Pharmacokinetics: Nicotinamide is a derivative of the B vitamin niacin. There appears to be no significant difference in the kinetics of low dose standard nicotinamide (2.5 mg/kg) and low-dose, long acting nicotinamide (Enduramide®) (6.7 mg/kg). Nonlinear kinetics is found with both formulations at higher doses. The FAUC is significantly greater with the standard formulation, indicating a higher bioavailability. The AUC for standard nicotinamide is 1.7 times higher than that for Enduramide®.

### Riboflavin

Erythrocytes lack mitochondria and other organelles and thus their cytoplasmic metabolism is much reduced. A small portion of the glucose transported into RBCs is metabolized via the glucose hexose-monophosphate pathway. The NADPH thus formed is important for processes involved in protecting RBCs from ROS. In addition to damaging intracellular molecules, cellular organelles and membranes, ROS convert hemoglobin into inactive methemoglobin.

Selenium-containing GSHPx converts peroxide groups into harmless hydroxyl units utilizing GSH as its substrate. The thiol group of the cysteine moiety is oxidized to the disulfide during the reduction of methemoglobin and peroxides. The regeneration of GSH is catalyzed by gluathione reductase, which in turn, uses NADPH as the reducing agent. The latter function is dependent on riboflavin.

The GSH content and glutathione reductase activity in the liver are decreased by deficiencies of riboflavin. However the riboflavin GSH content and activity of glutathione reductase returns to the control level of riboflavin-supplemented rats in 24 h and the lipid peroxide level recovers in 48 h. These findings indicate that increases of lipid peroxide in the livers of riboflavin-deficient rats is caused by the decrease in the GSH content as well as glutathione reductase activity rather than by decreases in the selenium-dependent GSHPx activity.

If the recommended daily of riboflavin intake is 0.5 mg/1 000 kcal (as is true), 23% of males and 7% of females are deficient in dietary riboflavin.

Absorption: Saturable (active transport) and nonsaturable (energy-independent) diffusion of riboflavin occur throughout the rat small intestine.

Pharmacokinetics: A small circadian variation in riboflavin levels occurs; plasma concentrations and urinary excretion of riboflavin are lowest during the afternoon. Since riboflavin has the potential to increase gastrointestinal iron absorption in the stomach, it is included in the delayed release portion of the combination dosage form of the invention to avoid this result.

### Selenium

Given the defined importance of the GSH/GSHPx system in preventing and treating NIHL, the necessity of adequate selenium (a GSHPx cofactor) is self-evident.

Selenium treatment results in a significant elevation of RBC GSHPx an increase in glutathione reductase activities and in GSH content by 64%, 57%, and 11%, respectively; this effect is also paralleled by a 39% reduction in the RBC oxidized GSII content. On termination of selenium treatment and after 3 months on placebo, all of these elements of the GSH system return toward baseline levels. Dietary selenium activates the GSH system and is thereby a potent antioxidant cofactor against plasma and LDL lipid peroxidation.

In mice, selenium supplementation increases GSH content and GSHPx activity in peritoneal macrophages by 36% and 30% respectively and this effect is associated with a 46% reduction in cell-mediated oxidation of LDL and aortic atherosclerotic lesions. These data demonstrate an inverse relationship between macrophage GSH content/GSHPx activity and cell-mediated oxidation of LDL and imply that enhancement of the macrophage GSH/GSHPx system contributes to attenuation of the atherosclerotic process.

Absorption: Sodium selenite is absorbed slowly, possibly by simple diffusion through the intestinal mucosa.

Pharmacokinetics: Thiols positively influence mucosal uptake of selenium. As an example, L-cysteine stimulates selenium uptake in the middle and distal jejunum and cecum, but not in the proximal jejunum. This effect is maximal in the distal jejunum. The absorption of amino acid-bound selenium is accelerated by specific amino acid active transport mechanisms in the gut mucosa.

### Taurine

We have established that $Ca^{+2}$ is a critically important intracellular messenger and that its intracellular signaling, occurring infrequency modulated waves, can be modified by calcium channel blockers which change the amplitude of $Ca^{+2}$ entering the cell. This modulated signaling requires an enzyme-controlled fine balance between the plasma and the endoplasmic reticular substances and $Ca^{+2}$ flow patterns within and from the cytoplasm. As described above, $Mg^{+2}$ is a key cofactor. Similarly, the amino acid taurine improves cellular $Ca^{+2}$ dynamics and is functionally complementary to $Mg^{+2}$.

Taurine is an amino acid, which is not utilized in protein synthesis, but rather is found free in the cytoplasm or in simple peptides. It is important for the modulation of cellular $Ca^{+2}$ levels, cell membrane stabilization and osmoregulation, and has been used with some success in the

treatment of neurodegenerative diseases, including macular degeneration and Alzheimer's disease. There is consistent evidence from other studies that taurine reduces toxic effects on neurons. While taurine increases cytosolic $Ca^{+2}$ transients in cardiac cells (and thus has positive inotropic activity), in other cells it tends to reduce cytosolic $Ca^{+2}$ consistent with its role as a modulator of $Ca^{+2}$ intracellular signaling. Similar to $Mg^{+2}$, taurine lowers elevated blood pressure, retards cholesterol-induced atherogenesis, prevents arrhythmias, and stabilizes platelets and cell membranes. Its favorable modulation of $Ca^{+2}$ signaling complements the similar action of $Mg^{+2}$ and its stabilization of cell membranes augments other components of this invention.

Absorption: Taurine uptake across the intestinal brush border membrane of the adult cat seems not to require a specific transport mechanism, although the steady-state uptake of taurine by rat intestinal cells is saturable.

Pharmacokinetics: Even at a low concentration taurine seems to enhance drug absorption—especially lipid soluble drugs—due to its effect on the permeability characteristics of the mucosal membrane. Bile salts are synthesized in the liver from cholesterol conjugated with taurine. Within the gastrointestinal lumen these bile salts play an essential role in lipid absorption and fat transport.

alpha-Tocopherol (See extended comments above regarding rheology.)

Improvements of Theological properties of blood and red cell deformability by alpha-tocopherol occur and are mainly attributed to reducing lipid peroxidation of red blood cell membranes. 300 mg tid of alpha-tocopherol taken after meals for 3 months results in significant reductions of blood viscosity at different shear rates and viscoelasticity. The resistance of erythrocyte deformation and lipid peroxidation stress in red cell membranc (malondialdchyde or MDA) is reduced by 0.17+/−0.13 nmol.

Fibronectin is significantly increased in patients with sensorineural hearing impairment, suggesting that microvascular endothelial damage is a factor in these patients. D, alpha-tocopherol is generally regarded as the most important lipid-soluble, chain-breaking antioxidant in maintaining the integrity of the vascular endothelial cells plasma

The lipid soluble, free oxygen radical scavenger, D, alpha-tocopherol has a variety of antioxidant activities which include among others: promotion of the Ach/cAMP synthesis of NO, decomposition of fatty acid hydroperoxides and hydrogen peroxides, maintenance of cell membrane stability, maintenance of RBC deformability, reduction of blood viscosity and viscoelasticity; prevention of DNA strand breakage, improvement in the uptake of glutamate by synaptosomes at neural junctions and the suppression of oxidation of oxLDL.

The antioxidant defense of elderly patients is improved with low doses of supplemental vitamin E, and following supplementation with D, alpha-tocopherol GSHPx activities increase as much as twofold. The importance of maintenance of GSH in reducing noise induced bearing loss has been addressed previously, as was its prevalence in the elderly.

Studies of the effectiveness of D, alpha-tocopherol in preventing or treating human pathology utilize dosages of 200 to 800 mg daily, which exceed amounts that can be obtained from an average diet and the curreot recommended daily allowance for healthy people. Thus supplementation at these levels is appropriate in therapeutics and disease prophylaxis.

Absorption: The gastrointestinal absorption of dietary D, alpha-tocopherol is dependent upon the simultaneous digestion and absorption of the fat in which the vitamin is solubilized. Taurinc may enhance D, alpha-tocopherol absorption. The site of D, alpha-tocopherol absorption is probably the proximal small intestine.

Pharmacokinetics: Evidence suggests that further uptake of the tocopherols occurs in the deep crystal zone of the colonic mucosa where actively proliferating cells extract nutrients from the systemic circulation.

Vanadium

Vanadium reduces insulin resistance. Insulin resistance precedes almost all Type 2 diabetes, which accounts for 95% of all diabetes mellitus.

Hearing loss is associated with diabetes mellitus. It is the most common causative metabolic disorder related to PSNHL. A hearing aid is three to four times more prevalent in patients with diabetes mellitus type 2 than in subjects without diabetes of the same age and about one-half of type 2 diabetic patients have impaired hearing.

The pathophysiologic importance of insulin resistance in diabetes mellitus has been established. Complexes of vanadium mimic the metabolic actions of insulin in improving glycemic control in humans with diabetes. In addition to its direct insulin mimetic actions, vanadium salts also modulate insulin metabolic effects by enhancing insulin sensitivity and prolonging insulin action. All of these actions appear to be related to protein tyrosine phosphatase (PTP) inhibition. The precise biochemical vanadate pathways of action are not yet known, but they are different from insulin in that the receptor for insulin and the enzyme phosphatidylinositol 3'-kinase do not seem to be essential for vanadate stimulation of glucose uptake and metabolism.

Vanadium can 'bypass' defects in insulin action in diseases characterized by insulin resistance. Vanadium salts also have an apparently preferential metabolic (versus mitogenic) signaling profile. These characteristics make vanadium compounds exciting pharmacological agents to reduce insulin resistance and improve diabetes associated hearing loss.

Vitamin A (beta-Carotene)

Vitamin A is found in the guinea pig cochlea at a concentration ten times that found in most other tissues. The effect of vitamin A deficiency in guinea pigs on noise-induced temporary threshold shift (TTS) was evaluated after short (15 min) acoustic over stimulation with a moderate (90 dB) broad-band white noise. Guinea pigs were fed, ad libitum, a purified diet deficient in vitamin A until biochemical signs of deficiency occurred. This resulted in caused a reduction in N1-amplitude and N1-latency in the vitamin A deficient, sound-stressed group; presumably this reflected changes in inner ear hair cell activity. The authors concluded that vitamin A deficiency increases the sensitivity of the inner ear to noise and that this increased sensitivity raises the probability of noise-induced hearing loss. In another study, after feeding young rats a diet deficient in vitamin A there were changes in the outer and inner hair cells and massive degenerative changes in the ganglion cells of the VIII nerve. In humans suffering from alcoholic liver disease there is decreased auditory function associated with low vitamin A levels. Studies also have shown some improvement in presbycusis in patients treated with vitamins A (and E) for 28–48 days.

Vitamin B6 (Pyridoxine)

Pyridoxine complements folic acid in reducing plasma homocysteine. Inducible iNOS (a Type II gene product)

within activated macrophages contributes to the inflammation that characterizes early atherogenesis and may, in part, account for the adverse vascular effects of hyperhomocysteinemia. Evidence suggests that the expression of iNOS in VSMCs may, in part, promote atherosclerosis by increasing local oxidative stress caused by high (toxic) local levels of NO. However, within activated macrophages the incorporation of pyridoxine (and of folic acid) lessens the conversion of L-arginine to toxic levels of homocysteine-induced NO from iNOS Type II. This adds an element of safety to the invention.

Absorption: Pyridoxine absorption in the jejunum (rat) is nonsaturable and consistent with passive diffusion. The gastrointestinal concentrations of pyridoxine in various intestinal segments tend to parallel those of riboflavin, suggesting some similarity of absorption characteristics. While the gastrointestinal absorption characteristics may be similar to riboflavin, it is unclear if it also enhances iron absorption like the latter.

Pharmacokinetics: Studies have suggested that a physiological dose of pyridoxine is transformed to pyridoxal in the intestinal tissues and then released in this putatively active form into the portal blood.

Vitamin $B^{12}$ (Cyanocobalamin)

There is convincing evidence that poor vitamin $B^{12}$ and folate status is associated with age-related auditory dysfunction. A thorough audiometric assessment was conducted in 55 healthy women aged 60–71 yrs. Hearing function was determined by the average of pure-tone air conduction thresholds at 0.5, 1, 2, and 4 kHz and was categorized into 2 groups for logistic regression analyses. The mean age was the same (65 y) for the normal and the impaired hearing groups. Pure-tone averages were inversely correlated with serum vitamin $B^{12}$ ($r=-0.58$, $P=0.0001$) and red cell folate ($r=-0.37$, $P=0.01$). Women with impaired hearing had 38% lower serum vitamin $B^{12}$ and 31% lower red cell folate than women with normal hearing. Among participants who did not take supplements containing vitamin $B^{12}$ or folate, women with impaired hearing had 48% lower serum vitamin $B^{12}$ and 43% lower red cell folate than women with normal hearing.

Absorption: The ileum is the major site of absorption of vitamin $B^{12}$ where its intestinal absorption is facilitated by two receptors and two transporters.

Pharmacokinetics: In nature, vitamin $B^{12}$ is only exceptionally met in its free form. It is almost always associated with a binder. Alimentary vitamin $B^{12}$ released from its protein complexes by culinary preparation and gastric secretions, is combined with haptocorrin. In the duodenum, haptocorrin is partially degraded by pancreatic enzymes and intraluminal pH and $B^{12}$ is attached to intrinsic factor for transfer. This combination of the vitamin can then be "caught" by the ileal receptor.

Vitamin D (calciferol)

Otosclerosis is a bone dysplasia limited to the otic capsule causing abnormal resorption and redeposition of bone. The existence of the entity "pure labyrinthine otosclerosis" or "cochlear otosclerosis" is not accepted by all authors; however, there is clinical and histologic evidence to support the existence of a progressive SNHL due to otospongiotic-otosclerotic lesions of the labyrinthine.

A number of authors confirm that vitamin D deficiency with its associated otosclerosis is one of the etiologies of PSNHL, although at least one study was not supportive of this view.

Treatment with vitamin D should prevent progressive hearing loss relating to otosclerosis and may in some cases improve hearing, which may be partly reversible. As an example, the degree of bone atrophy was quantitatively assessed by microdensitometry (MD) in 56 patients with senile deafness. Biochemical examinations were also made leading to a conclusion that abnormal bone metabolism is a factor in some patients with senile deafness. After treatment with an active vitamin D preparation (1 alpha-(OH)D3) at 1 microgram/day for 6 to 10 months, 12 patients suspected of having abnormal bone metabolism had hearing improvement in six ears (four patients).

Zinc ($Zn^{2+}$)

$Zn^{2+}$ deficiency is one cause of presbycusis. $Zn^{2+}$ supplementation in patients who are marginally $Zn^{2+}$ deficient, results in reduction of tinnitus and SNHL in about one-third of those who are elderly adults. While $Zn^{2+}$ supplementation may be useful in improving hearing loss, it must be used with care since this element is a neural transmitter and its use in excess can result in excitotoxicity.

Copper/zinc superoxide dismutase (Cu/$Zn^{2+}$ SOD) is a first-line defense against free radical damage in the cochlea and other tissues. Studies indicate that Cu/$Zn^{2+}$ SOD deficiencies increase the vulnerability of the cochlea to damage associated with normal aging, presumably through metabolic pathways involving the superoxide radical.

Glutamatergic receptors, as exist in the neural pathway related to hearing are vulnerable to excitotoxicity. In some circumstances, an excess of $Ca^{2+}$ influx alters neuronal metabolism and may become lethal for the cell. Two divalent cations, $Mg^{2+}$ and $Zn^{2+}$, have inhibitory effects on the involved NMDA receptors. $Mg^{2+}$ exerts a voltage-dependent block of the NMDA calcium channel, whereas $Zn^{2+}$ exerts a voltage-independent NMDA block. (Melatonin also has inhibitory effects on the NMDA receptor. In the rat, iontophoresis of melatonin, $Mg^{2+}$ and $Zn^{2+}$ produce a potent attenuation of the excitatory response of the cortical striatum, although the latency of the effect of melatonin was longer than those of $Mg^{2+}$ and $Zn^{2+}$. When these cations were simultaneously delivered with melatonin, additive inhibitory effects were recorded. These observations suggest that the inhibitory effects produced by the two cations and by melatonin are produced via different processes. The inhibitory role of melatonin on the NMDA receptor activity appears to be exclusive of a direct action on the NMDA calcium channel.

Absorption: Absorption of $Zn^{+2}$ ranges from 40 to 86%. About 37% of ingested $Zn^{+2}$ enters the plasma and gastrointestinal absorption is essentially completed by 4 hours. The duodenum and ileum are important sites for rapid $Zn^{20\ 2}$ absorption. A continuous, slower absorption of $Zn^{+2}$ may take place in the jejunum while the stomach, cecum and colon appear to be insignificant sites of absorption.

Pharmacokinetics: Mean plasma $Zn^{+2}$ increases only 37% above pre-load levels in face of an 11-fold increase in intake.

## DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

All terms appearing in this specification and the appended claims are used in the same manner as commonly recognized among those skilled in the technology and terminology of pharmacology. These terms are therefore used in

accordance with their conventional definitions, except as otherwise noted. Further clarifications of some of these terms as they apply specifically to this invention are offered below.

"Unit dosage form" refers to a composition intended for a single administration to treat a subject suffering from a disease or medical condition. Each unit dosage form typically comprises each of the active ingredients of this invention plus pharmaceutically acceptable excipients. Examples of unit dosage forms are individual tablets, individual capsules, bulk powders, and liquid solutions, emulsions or suspensions. Treatment of the disease or condition may require periodic administration of unit dosage forms, for example: one unit dosage form two or more times a day, one with each meal, one every four hours or other interval, or only one per day. The expression "oral unit dosage form" indicates a unit dosage form designed to be taken orally.

An "active agent"or "active ingredient" is a component of a dosage form that performs a biological function when administered or induces or affects (enhances or inhibits) a physiological process in some manner. "Activity" is the ability to perform the function, or to induce or affect the process. Active agents and ingredients are distinguishable from excipients such as carriers, vehicles, diluents, lubricants, binders, and other formulating aids, and encapsulating or otherwise protective components.

"Delivery vehicle" is a composition, which comprises one or more active agents, and is designed to release the active agent in a particular fashion, either by immediately dispersing the agents in the digestive system, or by releasing the agents in a slow sustained fashion. The term encompasses porous microspheres, microcapsules, cross-linked porous beads, and liposomes that contain one or more active ingredients sequestered within internal cavities or porous spaces. The term also includes osmotic delivery systems, coated tablets or capsules that include nonporous microspheres, microcapsules, and liposomes, and active agents dispersed within polymeric matrices. A dosage form can include one or more delivery vehicles.

"Controlled"or "sustained" or "time release" delivery are equivalent terms that describe the type of active agent delivery that occurs when the active agent is released from a delivery vehicle at an ascertainable and manipulatable rate over a period of time, which is generally on the order of minutes, hours or days, typically ranging from about thirty minutes to about 3 days, rather than being dispersed immediately upon entry into the digestive tract or upon contact with gastric fluid. A controlled release rate can vary as a function of a multiplicity of factors. Factors influencing the rate of delivery in controlled release include the particle size, composition, porosity, charge structure, and degree of hydration of the delivery vehicle and the active ingredient(s), the acidity of the environment (either internal or external to the delivery vehicle), and the solubility of the active agent in the physiological environment, i.e., the particular location along the digestive tract.

"Targeted" or "site-specific" delivery means that the pharmaceutical preparation is formulated to limit the release of its contents in an amount appropriate to the site where release occurs. The term refers in particular to the active agent, whose site-specific delivery implements the performance of the therapeutic function at a specific site within the body of the subject to whom the preparation is administered.

The phrase "therapeutically effective amount" means an amount sufficient to produce a therapeutic result. Generally the therapeutic result is an objective or subjective improvement of a disease or condition, achieved by inducing or enhancing a physiological process, blocking or inhibiting a physiological process, or in general terms performing a biological function that helps in or contributes to the elimination or abatement of the disease or condition.

"Vasoconstriction" is the reduction of the cross section of a blood vessel lumen, inhibiting the free flow of blood through the vessel. Vasoconstriction can arise from vasospasm, deposits on or in the lumen wall or from the thickening of the wall material due to excessive growth or proliferation of one or more of the wall layers.

The phrase "substantially homogeneous," when used to describe a formulation (or portion of a formulation) that contains a combination of components, means that the components, although each may be in particle or powder form, are fully mixed so that the individual components are not divided into discrete layers or form concentration gradients within the formulation.

L-ARGININE may be included in this invention as a free base or combined with the metallic cations contemplated by this invention —$Mg^{+2}$, $Cu^{+2}$ or $Zn^{+2}$- as metal L-arginine complexes which have the following formula:

$$[Arg]MX$$

wherein,

a. Arg is the amino acid L-arginine or bis-L,arginine;

b. M is a metal ion taken from, $Mg^{+2}$, $Cu^{+2}$ or $Zn^{+2}$;

c. X is an anion taken from the group including hydroxides, halides, sulfates, acetates, ascorbates or bis-ascorbic acid salts.

N-ACETYL-CYSTEINE (NAC), mercaptopropionylglycine (MPG) or L-2-oxothiazolidine-4-carboxylate (OTC) may be included in this invention as a free base or combined with the metallic cations contemplated by this invention —$Mg^{+2}$, $Cu^{+2}$ or $Zn^{+2}$-as metal complexes which have the following formula:

$$[A]MX$$

wherein,

a. A is cysteine, acetylcysteine, NAC, MPG or OTC;

b. M is a metal ion taken from the metallic cations contemplated by this invention: $Mg^{+2}$, $Cu^{+2}$ or $Zn^{+2}$,

c. X is an anion taken from the group including hydroxides, halides, sulfates, phosphates, acetates, ascorbates or bis-ascorbic acid salts.

alpha-LIPOIC ACID (LA) or thioctic acid (TA) may be included in this invention as a free base or combined with the metallic cations contemplated by this invention; $Mg^{+2}$, $Cu^{+2}$, $Zn^{+2}$ or $Se^{+2}$ as metal alpha-lipoic acid or thioctic acid complexes which have the following formula:

$$[A]MX$$

wherein,

a. A is LA or TA;

b. M is a metal ion taken from, $Mg^{+2}$, $Cu^{+2}$, $Zn^{+2}$ or selenium ($Se^{+2}$);

c. X is an anion taken from the group including hydroxides, halides, sulfates, phosphates, acetates, ascorbates or bis-ascorbic acid salts.

or

$$[A]_2MX$$

wherein,

a. A is LA or TA,

b. M is a metal ion taken from $Mg^{+2}$, $Cu^{+2}$, $Zn^{+2}$ or $Se^{+2}$;

c. X is an anion taken from the group including hydroxides, halides, sulfates, phosphates, acetates or ascorbates or bis-ascorbic acid salts.

L-ARGININE. N-ACETYL-CYSTEINE, TAURINE and alpha-LIPOIC ACID and metals of this invention; $Mg^{+2}$, $Cu^{+2}$, $Zn^{+2}$ or $Se^{+2}$ may also be included as bi-amide complexes in formulae of this invention with the following structure:

[A]MX

wherein,

a. A is 2,N-thioctylarginine (2NTA), 2,N-thioctylcysteine (2NTCy), or 2,N-thioctyltaurine (2NTT);

b. M is a metal ion taken from $Mg^{+2}$, $Cu^{+2}$, $Zn^{+2}$ or $Se^{+2}$;

c. X is an anion taken from the group including hydroxides, halides, sulfates, phosphates, acetates or ascorbates or bis-ascorbic acid salts,

or

$[A]_2MX$

wherein,

a. A is 2,N-thioctylarginine (2NTA), 2,N-thioctylcysteine (2NTCy), or 2,N-thioctyltatrine (2NTT);

b. M is a metal ion taken from $Mg^{+2}$, $Cu^{+2}$, $Zn^{+2}$ or $Se^{+2}$;

c. X is an anion taken from the group including hydroxides, halides, sulfates, phosphates, acetates or ascorbates or bis-ascorbic acid salts.

TAURINE may be used in this invention in its free forms or complexed or both. Absorption characteristics and pharmacokinetics are described in the "Solubility and Gastrointestinal Absorption Characteristics of the Components" (see above).

MAGNESIUM is present either as $Mg^{+2}$ salts or $Mg^{+2}$ complexes that release magnesium ion when ingested, or both. Examples of salts of $Mg^{+2}$ that can be used in this invention are acetate, acetyl-cysteinate, arginate, ascorbate, lipoate, malate, oxide, stearate, sulfate and taurate. As an example of kinetics after ingestion, magnesium ascorbate is soluble in gastric fluid and the respective components are absorbed in the gastrointestinal tract. The ascorbate radical serves as an adequate source of vitamin C by conversion to ascorbic acid upon exposure to hydrochloric acid in the gastric fluid, while the magnesium ion is converted to soluble magnesium chloride. The satisfactory water solubility of magnesium ascorbate provides for a diffusional gradient of $Mg^{+2}$ in the upper small intestine where some passive absorption of $Mg^{+2}$ occurs. Magnesium oxide is converted to magnesium chloride in the acid environment of the stomach and offers the advantage of high ionic magnesium content, since 60% by weight of the magnesium oxide molecule is $Mg^{+2}$. Magnesium stearate is useful as a lubricant when compressing the composition into tablets, in addition to its use as a minor $Mg^{+2}$ source. Preferred $Mg^{+2}$ sources include magnesium ascorbate, magnesium taurate, magnesium oxide or one of the complexes described previously.

ZINC is present either as $Zn^{+2}$ salts or $Zn^{+2}$ complexes that release $Zn^{+2}$ when ingested, or both. Absorption and pharmacokinetics are described above. Examples of salts of $Zn^{+2}$ that can be used in this invention are acetate, arginate, lipoate, sulfate, and taurate. Preferred $Zn^{+2}$ sources include zinc acetate, zinc taurate or one the complexes described previously.

SELENIUM is present either as $Se^{+2}$ salts or $Se^{+2}$ complexes that release selenium ion when ingested, or both. Absorption and pharmacokinetics are described above. Examples of salts of $Se^{+2}$ that can be used in this invention are acetate, arginate, lipoate, sulfate, and taurate. Preferred $Se^{+2}$ sources are L-selenomethionine, selenium from yeast or from the one of the complexes described previously.

CHROMIUM is present as $Cr^{+3}$ salts release chromium ion when ingested. Absorption and pharmacokinetics are described above. Preferred $Cr^{+3}$ sources include chromium tripicolinate or chromium binicotinate.

VANADIUM is present as vanadium ($V^{2+}$ to $V^{5+}$) salts and release vanadium ion when ingested. Absorption and pharmacokinetics are described above. Preferred vanadium sources include vanadyl sulfate or organic vanadium compounds, such as bis(maltolato)oxovanadium(IV).

D, alpha-TOCOPHEROL and its analogs and esters are D, alpha-tocopherol, D, alpha-tocopherol acid succinate, D, alpha-tocopherol nicotinate and D, alpha-tocopherol acetate. A particularly preferred form of vitamin E is D, alpha-tocopherol acid succinate or microencapsulated D, alpha-tocopherol nicotinate, especially for preparations in tablet form. The gastrointestinal absorption of dietary D, alpha-tocopherol is bile salt dependent and therefore is somewhat also dependent upon the simultaneous digestion and absorption of fat. The presence of dietary taurine, involved in the conversion of cholic acid to dexycholic acid in the gut, enhances D, alpha-tocopherol absorption. In these respects D, alpha-tocopherol absorption may be similar to that of vitamin A and the site of major vitamin A absorption is the proximal small intestine.

ASCORBATE is present either as ascorbic acid, metallo-ascorbate salts or complexes of ascorbate, or all of these. Examples of metallic salts of ascorbate that can be used in this invention are $Mg^{+2}$, $Cu^{+2}$ or $Zn^{+2}$. Absorption and pharmacokinetics are described above.

GINGKOLIDES (EGB), MELATONIN, UBIQUINONE and the B VITAMINS are present within this invention in their free forms. Absorption and pharmacokinetics are described above.

A slower, more sustained release of the active agents can be achieved by placing the active agents in one or more delivery vehicles that inherently retard the release rate. Examples of such delivery vehicles include polymeric matrices that maintain their structural integrity for a period of time prior to dissolving, or that resist dissolving in the stomach but are readily made available in the post-gastric environment by the alkalinity of the intestine, or by the action of metabolites and enzymes that are present only in the intestine. The preparation and use of polymeric matrices designed for sustained drug release is well known. Examples are disclosed in U.S. Pat. No. 5,238,714 (Aug. 24, 1993) to Wallace et al.; Bechtel, W., Radiology 161: 601–604 (1986); and Tice et al., EPO 0302582, Feb. 8, 1989. Selection of the most appropriate polymeric matrix for a particular formulation can be governed by the intended use of the formulation. Preferred polymeric matrices are hydrophilic, water-swellable polymers such as hydroxymethylcellulose, hydroxypropylcellulose, hydroxyethylcellulose, hydroxymethylpropylcellulose, polyethylene oxide, and porous bioerodible particles prepared from alginate and chitosan that have been ionically crosslinked.

A delayed, post-gastric, prolonged release of the active ingredients in the small intestine (duodenum, ileum, jejunum) can also be achieved by encasing the active agents, or by encasing hydrophilic, water-swellable polymers containing the active agents, in an enteric (acid-resistant) film. One class of acid-resistant agents suitable for this purpose is that disclosed in Eury et al., U.S. Pat. No. 5,316,774

("Blocked Polymeric Particles Having Internal Pore Networks for Delivering Active Substances to Selected Environments"). The formulations disclosed in this patent consist of porous particles whose pores contain an active ingredient and a polymer acting as a blocking agent that degrades and releases the active ingredient upon exposure to either low or high pH or to changes in ionic strength. The most effective enteric materials include polyacids having a $pK_a$ of from about 3 to 5. Examples of such materials are fatty acid mixtures, methacrylic acid polymers and copolymers, ethyl cellulose, and cellulose acetate phthalates. Specific examples are methacrylic acid copolymers sold under the name EUDRAGIT®, available from Rohm Tech, Inc., Maiden, Mass., USA; and the cellulose acetate phthalate latex AQUATERIC®, available from FMC Corporation, New York, N.Y., USA, and similar products available from Eastman-Kodak Co., Rochester, N.Y., USA.

Acid-resistant films of these types are particularly useful in confining the release of magnesium lactate and magnesium citrate to the post-gastric environment. Acid-resistant films can be applied as coatings over individual particles of the components of the formulation, with the coated particles then optionally compressed into tablets. An acid-resistant film can also be applied as a layer encasing an entire tablet or a portion of a tablet where each tablet is a single unit dosage form.

In certain embodiments of the invention, the dosage form is a substantially homogeneous single layer tablet that releases all of its components into the stomach upon ingestion (see below). In some embodiments a sustained dosage form is used and in others both dosage forms are combined into a bilayer tablet. Examples of the preferred ranges for components in each layer are shown in Table I.

TABLE I

| Component | Dosages in milligrams Preferred | Most Preferred | % in Bilayer Immed/Sustain | |
|---|---|---|---|---|
| Ascorbate | 75 to 3125 | 250 to 1250 | 50% | 50% |
| Calciferol | 30 to 1500 | 100 to 600 | 100% | |
| Carotene, beta | 30 to 1500 | 100 to 600 | 100% | |
| Chromium | 0.01 to 0.63 | 0.03 to 0.25 | 100% | |
| CoQ10 | 4.5 to 225 | 15 to 90 | 50% | 50% |
| Cyanocobalamin | 0.0006 to 0.010 | 0.002 to 0.004 | 100% | |
| Folate, Tetrahydro | 0.03 to 2.0 | 0.10 to 0.80 | 100% | |
| Ginkgo biloba | 7.5 to 250 | 25 to 100 | 50% | 50% |
| Glutathione | 30 to 1500 | 100 to 600 | 100% | |
| L-Arginine | 75 to 6250 | 250 to 2500 | 75% | 25% |
| Lipoate | 30 to 1500 | 100 to 600 | 50% | 50% |
| Magnesium | 30 to 1000 | 100 to 400 | 40% | 60% |
| Melatonin | 0.15 to 7.5 | 0.5 to 3 | 40% | 60% |
| N-Acetyl-L-Cysteine | 78 to 3900 | 200 to 1200 | 75% | 25% |
| Nicotinamide | 3.0 to 1500 | 10 to 150 | 25% | 75% |
| Pyridoxine | 0.3 to 15 | 1.0 to 6.0 | 100% | |
| Riboflavin | 3.6 to 188 | 12 to 75 | 50% | 50% |
| Selenium | 0.015 to 0.75 | 0.05 to 0.3 | 100% | |
| Taurine | 75 to 3125 | 250 to 1250 | 75% | 25% |
| Tocopherol, D alpha | 15 to 1600 | 50 to 800 | 100% | |
| Vanadium | 7.5 to 375 | 25 to 150 | 100% | |
| Zinc | 1.5 to 80 | 5 to 32 | 25% | 75% |

The dosage forms of the invention optionally include one or more suitable and pharmaceutically acceptable excipients, such as ethyl cellulose, cellulose acetate phthalates, mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, carbonate, and the like. These excipients serve a variety of functions, as indicated above, as carriers, vehicles, diluents, binders, and other formulating aids. In general, the dosage forms of this invention include powders, liquids, emulsions, tablets, transmembrane delivery systems, electrophoretic delivery systems and capsules.

The dosage forms of this invention can be formulated for administration at rates of two or more unit dosage forms per day. Tableted unit dosage forms to be taken three to four times per day are preferred.

The following example is offered for purposes of illustration only.

## EXAMPLE I

A single layer tablet, substantially homogenous in composition, which will disintegrate upon ingestion to provide simultaneous accessibility to all components, is prepared with the following composition:

TABLE II

| FOR RELEASE IN THE STOMACH | | 100% % of formula |
|---|---|---|
| $Mg(C_6H_7O_6)_2$ | Magnesium L-Ascorbate | 16.64% |
| $Mg(C_5H_9NO_3S)_2$ | Magnesium L-Acetylcysteine | 20.90% |
| $C_8H_{12}O_2S_2$ | Magnesium Lipoate | 5.18% |
| MgO | Magnesium Oxide | 12.64% |
| $C_{35}H_{53}N_{O3}$ | D a-Tocopherol Nicotinate | 17.15% |
| $C_{19}H_{19}N_7O_6$ | Folic acid | 0.012% |
| $C_5H_{11}NO_2Se$ | L-Selenomethionine | 0.007% |
| $C_{17}H_{20}N_4O_6$ | Riboflavin | 0.39% |
| $Mg(C_{18}H_{35}O_2)_2$ | Magnesium Stearate | 0.77% |
| . . . | Starch | 25.29% |

### Methods of Administration and Types of Utility

The compositions and dosage forms of the invention are useful for treating conditions commonly associated with hearing loss and tinnitus. The carefully chosen active ingredients of the invention act in a well-defined and complementary biochemical partnership to ensure that conditions of potential vascular risk are reduced in these patients. The resulting improvement in systemic and VIIIth nerve vascular health, especially in vascular endothelial health, maximizes the potential for avoiding hearing loss and deafness because of neglected, unrecognized or unappreciated ocular vascular inadequacy. The age group most commonly first diagnosed with hearing loss and tinnitus also is the age group moving into the physiological arena of reduced cellular efficiency secondary to age; at the same time it faces a concomitant increasing incidence of generalized vascular disability and associated chronic disease (e.g., diabetes mellitus, hypertension, hyperlipidosis, hyperinsulinemia). Many of the latter pathologies are associated with progressively widespread and worsening vascular health, a situation that threatens not only hearing but overall health.

By positively influencing the NO/ET-1 balance, reducing adverse homocysteine effects, modulating a controlled reduction in $Ca^{+2}$ cellular inflow via physiological calcium channel blockade, reducing platelet aggregation, lowering microviscosity and improving vascular laminar fluid dynamics, reducing the inflammatory risks associated with local free radicals such as hydroxyls and limiting the rate the oxidation of low-density lipids, the invention provides significant protection for patients. In performing these tasks, it positively influences conditions which otherwise represent risks associated with sensorineural hearing loss and tinnitus.

Epidemiological studies have confirmed repeatedly that inadequate dietary intake of $Mg^{+2}$, ascorbate and folic acid,

among others, is common in the general, apparently healthy public and is especially rampant in alcoholics, institutionalized patients, cigarette smokers and the elderly. Other patients have disturbances of reduced absorption or abnormal loss of these and other critical biofactors (e.g., hypochlorhydria, diabetes mellitus, hyperinsulinemia, renal pathology, small or large bowel pathology, etc.) Another subset of patients suffers from a variety of primary diseases that create an underlying foundation of vascular dysfunction, which is worsened by coexistent deficiencies (e.g., essential hypertension, congenital dyslipogenesis, aging, diabetes mellitus type 1 or type 2, etc.) The distribution of patients at risk of hearing loss among any of the above groups is no less than in the general public and the passage of time subjects everyone to the debilitations of aging. The invention is especially useful in reducing the risks of hann associated with those various conditions of vascular dysfunction congruent with these events. While it should be expected that an improvement in general vascular health would be universally beneficial to all of these clinical groups, this invention focuses upon reducing conditions of risk or failing function associated with hearing loss.

The foregoing is offered primarily for purposes of illustration. It will be readily apparent to those skilled in the art that the proportions, materials, formulation procedures, administration protocols and other parameters of this invention may be farther modified or substituted in various ways without departing from the spirit and scope of the invention.

We claim:

**1**. A unit dosage form for the amelioration of adverse conditions or functions associated with hearing loss or tinnitus comprising as active ingredients:

(a) magnesium,

(b) D, alpha-tocopherol,

(c) folate,

(d) a thiol-containing glutathione-increasing agent,

(e) selenium,

(f) riboflavin,

(g) ascorbate, and

(h) zinc.

**2**. The unit dosage form of claim **1** in which:

(a) said magnesium is in the form of magnesium oxide in an amount ranging from about 60 mg to about 2500 mg and magnesium lipoate in an amount ranging from about 30 mg to about 1500 mg,

(b) said alpha-tocopherol is in the form of D, alpha-tocopherol nicotinate in an amount ranging from about 85 mg to about 3500 mg,

(c) said folate is present in an amount ranging from about 0.03 mg to about 2.0 mg,

(d) said thiol-containing glutathione-increasing agent is magnesium L-acetyl cysteine in an amount ranging from about 80 mg to about 4000 mg,

(e) said selenium is in the form of selenomethionine in an amount ranging from about 0.04 to about 1.0 mg,

(f) said riboflavin is in an amount of about 3.6 mg to about 188 mg,

(g) said ascorbate is in the form of magnesium ascorbate in an amount ranging from about 80 mg to about 4000 mg, and

(h) said zinc is in the form of zinc picolinate in an amount ranging from about 7.1 mg to about 500 mg.

**3**. The unit dosage form of claim **1** in which said active ingredients are formulated as a substantially homogeneous tablet that releases all of said active ingredients into the stomach upon ingestion and contact with gastric fluid.

**4**. The unit dosage form of claim **2** further comprising as active ingredients L-arginine in an amount ranging from about 75 mg to about 6300 mg and pyridoxine in an amount ranging from about 0.3 mg to about 15 mg.

**5**. The unit dosage form of claim **4** further comprising as active ingredients taurate in the form of magnesium taurate, in an amount ranging from about 75 mg to about 3100 mg, melatonin in an amount ranging from about 0.15 mg to about 7.5 mg and CoQ10 in an amount ranging from about 4.5 mg to about 225 mg.

**6**. A unit dosage form for the amelioration of adverse conditions giving rise to hearing loss and tinnitus, said unit dosage form comprising as active ingredients:

a thiol-containing glutathione-increasing agent having the formula

$$R_nMX$$

in which:

R is a member selected from the group consisting of N-acetyl-L-cysteine, L-2-oxothiazolidine-4-carboxylate, and N-2(-mercaptopropionyl)-glycine,

n is 1 or 2,

M is a member selected from the group consisting of $Mg^{+2}$, $Cu^{+2}$, $Zn^{+2}$, and $Se^{+2}$, and

X is a member selected from the group consisting of hydroxide, halide, sulfate, acetate, ascorbate, and bis-ascorbate.

**7**. The unit dosage form of claim **2** in which said zinc is in the form of zinc dinicotinate.

**8**. The unit dosage form of claim **2** in which said zinc is in the form of zinc ascorbate and is present in an amount ranging from about 9.5 mg to about 500 mg.

**9**. The unit dosage form of claim **2** in which said zinc is in the form of zinc L-acetylcysteinate and is present in an amount ranging from about 9 mg to about 480 mg.

**10**. The unit dosage form of claim **5** further comprising as active ingredients chromium in an amount ranging from about 0.1 mg to about 0.63 mg, cyanocobalamine in an amount ranging from about 0.0006 mg to about 0.01 mg and vanadium in an amount ranging from about 7.4 mg to about 375 mg.

**11**. The unit dosage form of claim **10**, further comprising as active ingredients biotin in an amount ranging from about 0.1 mg to about 0.20 mg, beta carotene in an amount ranging from about 30 mg to about 1500 mg, calciferol in an amount ranging from about 100 mg to about 600 mg and Gingko biloba in an amount ranging from about 7.5 mg to about 250 mg.

**12**. A unit dosage form for the amelioration of adverse conditions giving rise to hearing loss and tinnitus, said unit dosage form comprising as active ingredients:

(a) magnesium,

(b) copper,

(c) zinc, and

(d) selenium,

at least one of which is in the form of a complex having the formula

$$R_nMX$$

in which:

R is a member selected from the group consisting of 2,N-thiocty lcysteine, and 2,N-thiocty ltaurine,

n is 1 or 2,

M is a member selected from the group consisting of $Mg^{+2}$, $Cu^{+2}$, $Zn^{+2}$, and $Se^{+2}$, and

X is a member selected from the group consisting of hydroxide, halide, sulfate, acetate, ascorbate, and bis-ascorbate.

13. A layered tablet for the amelioration of adverse conditions associated with hearing loss and conditions giving rise thereto, said layered tablet comprising an immediate-release layer and a sustained-release layer, and comprising the following as active ingredients distributed between said immediate-release layer and said sustained-release layer in the following approximate proportions expressed as relative weight percents:

| | Immediate-Release Layer | Sustained-Release Layer |
|---|---|---|
| Magnesium | 40–60% | balance |
| Ascorbate | 100% | |
| alpha, tocopherol | 100% | |
| alpha, lipoic acid | 40–60% | balance |
| L-arginine | 40–60% | balance |
| beta, carotene | | 100% |
| Copper | 100% | |
| CoQ10 | 40–60% | balance |
| Chromium | | 100% |
| Nicotinamide | 100% | |
| Glutathione-increasing agent | 40–60% | balance |

14. A layered tablet for use as an oral dosage form, said layered tablet comprising an immediate-release layer and a sustained-release layer, and comprising the following as active ingredients distributed between said immediate-release layer and said sustained-release layer in the following approximate proportions expressed as relative weight percents:

| | Immediate-Release Layer | Sustained-Release Layer |
|---|---|---|
| Magnesium | 40–60% | balance |
| Selenium | 100% | |
| Vitamin D | 40–60% | balance |
| $L_1$ arginine | 40–60% | balance |
| Taurine | 40–60% | balance |
| Zinc | 100% | |
| Folic acid | 100% | |
| Gingko biloba | | 100% |
| Melatonin | 100% | |

* * * * *

# EXHIBIT F

# MICRONUTRIENT STATUS IN PATIENTS WITH GASTROINTESTINAL DISEASE

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## INTRODUCTION

Clinical signs of macronutrient deficiency such as weight loss, muscle wasting, and hypoproteinemia are often present in patients experiencing chronic gastrointestinal disease. Pathological processes that impair the digestion and absorption of protein, fat and carbohydrate also can affect the absorption of micronutrients. Which micronutrients might be affected depends several factors including the type of disease process, the anatomic location involved, and the severity and chronicity of the disease. For many micronutrients the signs of deficiency are subtle and nonspecific. In addition, determining whether a patient is experiencing a deficiency of any given vitamin or mineral is often complicated by the lack of a simple diagnostic test for determining nutrient status. Serum levels do not necessarily reflect tissue levels or whole body status. This is particularly the case with trace elements. Furthermore, even when determining a serum concentration is a reliable test, generally it is performed at only a limited number of laboratories and results will not be immediately available. Therefore, the clinician needs to keep in mind the potential for a micronutrient deficiency to occur and to pro-actively investigate nutrient status when circumstances dictate.

Many vitamins and minerals are absorbed throughout the gastrointestinal tract. A few, however, are absorbed in very specific sites and therefore localized disease or resection could lead to a deficiency of one of these. The duodenum and proximal jejunum are the principle sites of absorption of calcium, selenium, and vitamin $B_2$ (riboflavin). While iron can be absorbed throughout the intestine, the duodenum is the site of most efficient absorption. Folate is absorbed primarily in the upper third of the jejunum. The ileum is the only segment of the intestine where vitamin cobalamin ($B_{12}$) is absorbed. The ileum is also the site of bile acid reabsorption. Since feline bile acids are exclusively conjugated with the essential amino acid taurine, situations where the ileum is resected and bile acids are lost in the stool could conceivably lead to a taurine deficiency.

The type of underlying gastrointestinal disease will affect which type of micronutrients may be malabsorbed. Since the fat soluble vitamins, A, D, E, and K, can only be absorbed with long chain fatty acids, any defect in fat digestion (pancreatic or biliary secretion) or absorption (biliary secretion or mucosal disease) can lead to a deficiency of any or all of these vitamins. Fat malabsorption can also lead to malassimilation of calcium and magnesium due to the binding of these minerals by unabsorbed fatty acids. Chronic diarrhea can lead to losses of electrolytes, zinc, and other water-soluble trace elements. Pancreatic disease may lead to deficient secretion of intrinsic factor that is necessary for cobalamin absorption. And generalized mucosal disease can impair absorption of many vitamins and minerals.

## MICRONUTRIENT DEFICIENCIES RECOGNIZED IN CANINE AND FELINE GI DISEASE

### Cobalamin

Determination of serum cobalamin concentration has been useful in identifying and characterizing intestinal and pancreatic disease in dogs. Cobalamin homeostasis is a complex, multi-step process that involves participation of the stomach, pancreas, intestines and liver. Following ingestion, cobalamin is released from food in the stomach. It is then bound to a non-specific cobalamin-binding protein of salivary and gastric origin called haptocorrin. Intrinsic factor (IF), a cobalamin binding protein that promotes cobalamin absorption in the ileum, is produced by the stomach and pancreas in dogs, and the pancreas, but not the stomach, in the cat. The affinity of cobalamin for haptocorrin is higher at acid pH than for IF, so most is bound to haptocorrin in the stomach. Upon entering the duodenum haptocorrin is degraded by pancreatic proteases, and cobalamin is transferred from haptocorrin to IF, a process facilitated by the high affinity of IF for cobalamin at neutral pH. Cobalamin-IF complexes traverse the intestine until they bind to specific receptors (previously called IFCR, but recently dubbed cubilin) located in the microvillus pits of the apical brush-border membrane of ileal enterocytes. Cobalamin is then transcytosed to the portal bloodstream and binds to a protein called transcobalamin 2(TC II) which mediates cobalamin absorption by target cells. A portion of cobalamin taken up by hepatocytes is rapidly (within an hour in the dog) re-excreted in bile bound to haptocorrin. Cobalamin of hepatobiliary origin, in common with dietary derived cobalamin, undergoes transfer to IF and receptor mediated absorption, thus establishing enterohepatic recirculation of the vitamin. Low serum cobalamin concentrations in dogs have been associated with exocrine pancreatic insufficiency (EPI), severe intestinal disease and putatively small intestinal bacterial overgrowth. Where low serum cobalamin is detected and EPI and small intestinal bacterial overgrowth have been excluded, localization of the problem to the ileum can be inferred. Selective cobalamin malabsorption and cobalamin deficiency has been recognized in Giant Schnauzers with defective localization of the ileal cobalamin-intrinsic factor receptor. Cobalamin is an essential cofactor for the activity of methylmalonyl-CoA mutase and methionine synthase. Reduced activity of these two enzymes cause the biochemical signatures of cobalamin deficiency, methylmalonicacidemia/uria (MMA) and homocystelnemia/uria respectively. Affected Schnauzers have inappetance, failure to thrive, anemia, leukopenia and methylmalonyl aciduria, that are completely reversed by the parenteral administration of cobalamin.

Cobalamin deficiency has rarely been reported in the cat though recent observations indicate serum concentrations can be subnormal in cats with EPI, intestinal, pancreatic or hepatic disease. Forty-nine of 80 serum samples submitted from cats with signs of gastrointestinal disease during the period of January 1996-January 1998 had cobalamin concentrations below the reference range for healthy cats (range 900 - 2,800 pg/ml; mean ± SD = 1775 ± 535 pg/ml SD; n=33). Cats with subnormal cobalamin concentrations (mean ± SD = 384 ± 272 pg/ml, range 3 - 883pg/ml) were middle aged or older and were presented for weight loss, diarrhea, vomiting, anorexia and thickened intestines. Definitive diagnoses in 22 cats included inflammatory bowel disease, intestinal lymphoma, cholangiohepatitis or cholangitis, and pancreatic inflammation. Serum concentrations of cobalamin were particularly low

# EXHIBIT G

US006228367B1

(12) **United States Patent**
**Watson**

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(54) **FOOD SUPPLEMENT FORMULATION**

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(58) **Field of Search** ..... 424/523, 554, 424/555, 195.1; 435/134, 135; 426/585, 601, 615, 643

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(57) **ABSTRACT**

A food supplement formulation comprises flaxseed oil, borage seed oil, fish oil, and lipase.

**20 Claims, No Drawings**

# FOOD SUPPLEMENT FORMULATION

## FIELD OF THE INVENTION

The present invention relates generally to a food supplement formulation. More particularly, the invention is directed to a food supplement formulation primarily containing essential fatty acids which are important for maintaining good health.

## BACKGROUND OF THE INVENTION

Natural compounds and herbal formulations can provide a supplement to the daily human diet. Certain compounds are useful to the human body, but are not produced in substantial quantities thereby. Thus, natural formulations have been found to be useful for supplementing the intake of these compounds from the human diet.

It would be desirable to prepare a food supplement formulation which may be taken in excess of the daily human diet, which food supplement formulation may promote general health.

## SUMMARY OF THE INVENTION

Accordant with the present invention, there surprisingly has been discovered a food supplement formulation, comprising:

flaxseed oil; borage seed oil; fish oil; and

lipase.

The food supplement formulation according to the present invention is useful as a dietary supplement.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention is directed to a food supplement formulation, comprising flaxseed oil, borage seed oil, fish oil, and lipase. The inventive formulation may be mixed together by conventional mixing equipment, and inserted, in dosage-sized quantities, into gelatin capsules for oral administration.

Flaxseed oil is a well-known compound containing omega-6 and omega-3 essential fatty acids in the forms of alpha-linolenic acid and linoleic acid. The body converts these fatty acids into other important fatty acids, which are used by the body for the production of prostaglandins. Prostaglandins are then used by the body to maintain healthy cholesterol and blood fat levels, support healthy blood pressure levels, and protect the membranes that surround the body's nerves.

Flaxseed oil may be present in the inventive food supplement formulation at a concentration from about 10 to about 60 weight percent. Preferably, the concentration is from about 20 to about 40 weight percent. Most preferably, the concentration of flaxseed oil is about 33.15 weight percent.

Borage seed oil is a well-known compound which contains a high concentration of the essential fatty acid gamma-linolenic acid. Gamma-linolenic acid is normally synthesized in the liver from dietary linoleic acid. The synthesis is deficient in a substantial number of people because of the interference by sugar, saturated fats, and trans-fatty acids. Gamma-linolenic acid is a precursor to the production of prostaglandins and other hormones in the body.

Borage seed oil may be present in the inventive food supplement formulation at a concentration from about 10 to about 60 weight percent. Preferably, the concentration is from about 20 to about 40 weight percent. Most preferably, the concentration of borage seed oil is about 33.15 weight percent.



Fish oil is a well-known compound which contains high concentrations of eicosapentaenoic acid and docosahexaenoic acid. These polyunsaturated long-chain fatty acids have been shown to assist in preventing cardiovascular disease, by reducing triglycerides and cholesterol in the blood stream, thinning the blood, and increasing the high-density lipoprotines in the body.

Fish oil may be present in the inventive food supplement formulation at a concentration from about 10 to about 60 weight percent. Preferably, the concentration is from about 20 to about 40 weight percent. Most preferably, the concentration of fish oil is about 33.15 weight percent.

Lipase is a well-known compound, consisting of enzymes that help the body's digestive system break-down and digest fats, cellulose, carbohydrates, and proteins. Lipase enzymes are produced by the body's liver and pancreas. In a substantial number of people, however, the production of lipase enzymes is deficient. Lipase from plants may be used to supplement the body's production.

Lipase may be present in the inventive food supplement formulation at a concentration from about 0.1 to about 2 weight percent. Preferably, the concentration is about 0.25 to about 1 weight percent. Most preferably, the concentration of lipase is about 0.55 weight percent.

The ingredients of the inventive food supplement formulation may synergistically work together to improve bodily functions such as, for example, cardiovascular function, joint flexibility, fat metabolism, nervous system and brain function, hormone production, and cell division.

Conveniently, the inventive food supplement formulation may be taken orally at a dosage rate ranging from about 200 milligrams per day to about 2,000 milligrams per day. Preferably, the dosage rate is about 1,000 milligrams per day. The prescribed dosage rates may be effective to supplement the lack of important compounds required by the body for promoting general health.

This invention is more easily comprehended by reference to the specific embodiments recited hereinabove which are representative of the invention. It must be understood, however, that the specific embodiments are provided only for the purpose of illustration, and that the invention may be practiced otherwise than as specifically illustrated without departing from its spirit and scope.

What is claimed is:

1. A food supplement formulation, consisting essentially of:

flaxseed oil;

borage seed oil;

fish oil; and

lipase.

2. The food supplement formulation according to claim 1, wherein the concentration of flaxseed oil ranges from about 10 to about 60 weight percent.

3. The food supplement formulation according to claim 1, wherein the concentration of borage seed oil ranges from about 10 to about 60 weight percent.

4. The food supplement formulation according to claim 1, wherein the concentration of fish oil ranges from about 10 to about 60 weight percent.

5. The food supplement formulation according to claim 1, wherein the concentration of lipase ranges from about 0.1 to about 2 weight percent.

6. The food supplement formulation according to claim 2, wherein the concentration of flaxseed oil ranges from about 20 to about 40 weight percent.

7. The food supplement formulation according to claim 3, wherein the concentration of borage seed oil ranges from about 20 to about 40 weight percent.

8. The food supplement formulation according to claim 4, wherein the concentration of fish oil ranges from about 20 to about 40 weight percent.

9. The food supplement formulation according to claim 1, wherein the concentration of flaxseed oil is about 33.15 weight percent, the concentration of borage seed oil is about 33.15 weight percent, the concentration of fish oil is about 33.15 weight percent, and the concentration of lipase is about 0.55 weight percent.

10. A food supplement formulation, consisting essentially of:

from about 10 to about 60 weight percent flaxseed oil;

from about 10 to about 60 weight percent borage seed oil;

from about 10 to about 60 weight percent fish oil; and

from about 0.1 to about 2 weight percent lipase.

11. The food supplement formulation according to claim 10, wherein the concentration of flaxseed oil ranges from about 20 to about 40 weight percent.

12. The food supplement formulation according to claim 10, wherein the concentration of borage seed oil ranges from about 20 to about 40 weight percent.

13. The food supplement formulation according to claim 10, wherein the concentration of fish oil ranges from about 20 to about 40 weight percent.

14. The food supplement formulation according to claim 10, wherein the concentration of lipase ranges from about 0.25 to about 1 weight percent.

15. The food supplement formulation according to claim 11, wherein the concentration of flaxseed oil is about 33.15 weight percent.

16. The food supplement formulation according to claim 12, wherein the concentration of borage seed oil is about 33.15 weight percent.

17. The food supplement formulation according to claim 13, wherein the concentration of fish oil is about 33.15 weight percent.

18. The food supplement formulation according to claim 14, wherein the concentration of lipase is about 0.55 weight percent.

19. A food supplement formulation, consisting essentially of:

from about 20 to about 40 weight percent flaxseed

from about 20 to about 40 weight percent borage seed oil;

from about 20 to about 40 weight percent fish oil; and

from about 0.25 to about 1 weight percent lipase.

20. A food supplement formulation, consisting essentially of:

about 33.15 weight percent flaxseed oil;

about 33.15 weight percent borage seed oil;

about 33.15 weight percent fish oil; and

about 0.55 weight percent lipase.

* * * * *

# EXHIBIT H

US006160007A

# United States Patent [19]

DeMichele et al.

[11] Patent Number: 6,160,007

[45] Date of Patent: *Dec. 12, 2000

[54] METHOD FOR ENHANCING THE ABSORPTION AND TRANSPORT OF LIPID SOLUBLE COMPOUNDS USING STRUCTURED GLYCERIDES

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[73] Assignee: Abbott Laboratories, Abbott Park, Ill.

[ * ] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: 09/388,331

[22] Filed: Sep. 1, 1999

Related U.S. Application Data

[63] Continuation of application No. 08/991,503, Dec. 16, 1997, Pat. No. 6,013,665.

[51] Int. Cl.' ..... A61K 31/355; A61K 31/07; A61K 47/00

[52] U.S. Cl. ..... 514/458; 514/725; 514/786; 514/946

[58] Field of Search ..... 514/458, 725, 514/786, 946

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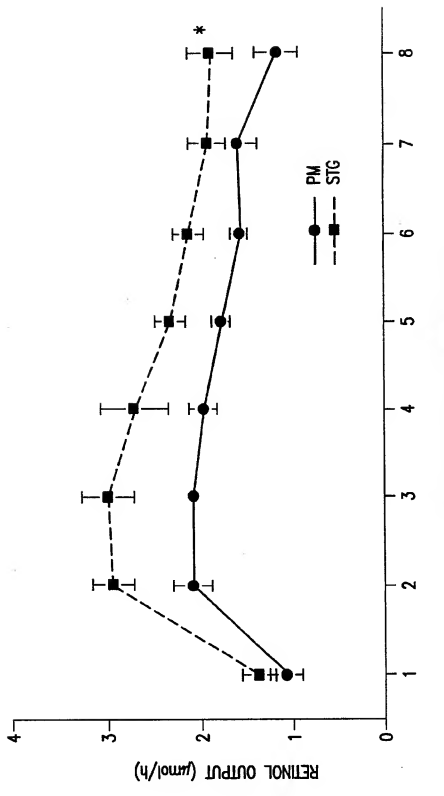
*Primary Examiner*—Kimberly Jordan
*Attorney, Agent, or Firm*—J. Michael Dixon

[57] ABSTRACT

This invention relates to a method for enhancing the absorption of oil soluble (lipophilic) compounds such as oil soluble vitamins, hormones, nutrients and drugs in an animal. The inventive method comprises administering a lipophilic compound in conjunction with a structured glyceride component characterized in that at least 40% of the glyceride species have: (i) about 33 to 70 wt. % of acyl moieties having 4 to 12 carbon atoms; (ii) about 30 to 67 wt. % of acyl moieties having more than 12 carbon atoms; and (iii) an equivalent carbon number greater than 30 and less than 48. This invention also relates to compositions suitable for administrating to an animal comprising a lipophilic compound and a structured glyceride component characterized in that at least 40% of the glyceride species have: (i) about 33 to 70 wt. % of acyl moieties having 4 to 12 carbon atoms; (ii) about 30 to 67 wt. % of acyl moieties having more than 12 carbon atoms; and (iii) an equivalent carbon number greater than 30 and less than 48. The method and compositions of the invention are especially suited for animals that suffer from lipid malabsorption conditions such as Crohn's disease, Cystic fibrosis, and short bowel syndrome.

98 Claims, 5 Drawing Sheets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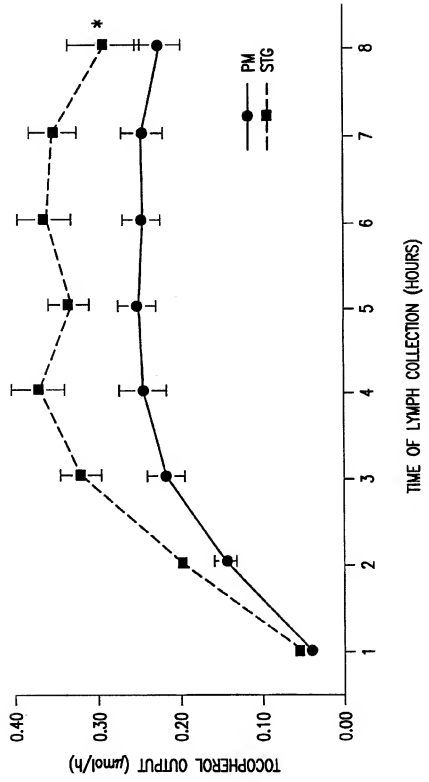
0
1
2
3
4
TIME OF LYMPH COLLECTION (HOURS)
1
2
3
4
5
6
7
8
PM
STG
*

FIG.1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0.20
0.30
0.40
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4
5
6
7
8
TIME OF LYMPH COLLECTION (HOURS)
PM
STG
*

FIG.2

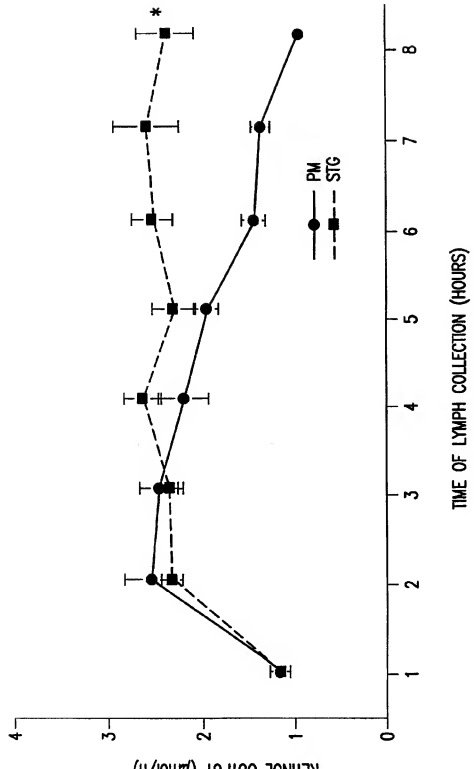
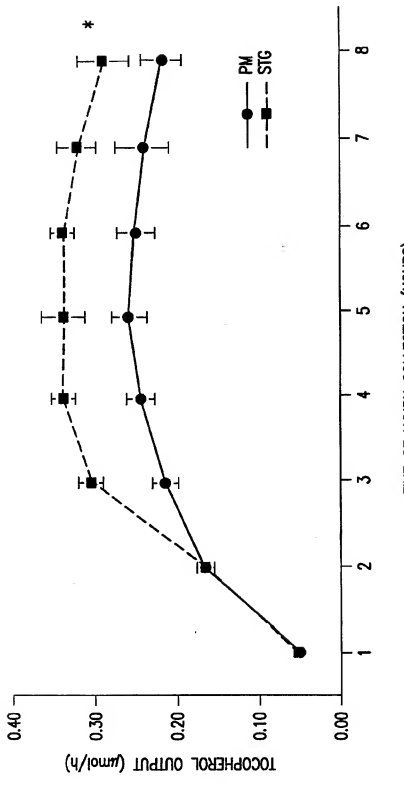

RETINOL OUTPUT (μmol/h)
0
1
2
3
4
1
2
3
4
5
6
7
8
TIME OF LYMPH COLLECTION (HOURS)
PM
STG
*

FIG.3


TOCOPHEROL OUTPUT (μmol/h)
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0.30
0.20
0.10
0.00
1
2
3
4
5
6
7
8
TIME OF LYMPH COLLECTION (HOURS)
PM
STG
*

FIG.4

FASTED MALE SPRAGUE-DAWLEY RATS (300 g)
CANNULATION OF MESENTERIC LYMPH DUCT AND GASTRIC TUBE INSERTION
SHAM-OPERATED CONTROLS
1 ml INTRAGASTRIC INFUSION
14C-TOCOPHEROL
3H-RETINOL
FISH OIL/MCT STRUCTURED LIPID n=6-7
FISH OIL/MCT PHYSICAL MIX n=6-7
ISCHEMIA/REPERFUSION 20 min. OCCLUSION OF S.M.A.
1 ml INTRAGASTRIC INFUSION
14C-TOCOPHEROL
3H-RETINOL
FISH OIL/MCT STRUCTURED LIPID n=6-7
FISH OIL/MCT PHYSICAL MIX n=6-7

FIG.5

# METHOD FOR ENHANCING THE ABSORPTION AND TRANSPORT OF LIPID SOLUBLE COMPOUNDS USING STRUCTURED GLYCERIDES

This application is a continuation of U.S. patent application Ser. No. 08/991,503, filed Dec. 16, 1997, which is now U.S. Pat. No. 6,013,665.

This invention relates to a method for enhancing the absorption and transport of lipid soluble compounds such as certain vitamins, nutrients and drugs in an animal. The inventive method comprises the administration of one or more lipid soluble compounds in conjunction with a structured glyceride component containing at least 33 wt. % of a fatty acid moiety having 4 to 12 carbon atoms,. at least 30 wt. % of a fatty acid moiety having more than 12 carbon atoms and an equivalent carbon number (ECN) greater than 30 to less than 48. This invention also relates to compositions comprising a structured glyceride component and one or more lipid soluble compounds. The most preferred compositions for use in this invention are suitable for enteral administration to an animal.

## BACKGROUND

Lipoidal preparations have been extensively studied in an effort to improve drug absorption from the gastrointestinal tract. Mammals with lipid (fat) malabsorption diseases such as Cystic fibrosis, Crohn's disease, Short bowel syndrome and the like, present special problems for the digestion, absorption and lymphatic transport of dietary fat and lipophilic compounds, such as drugs, hormones, nutrients and vitamins since the underlying disease limits the absorption and transport of the lipophilic compound from the gut. Absorption of lipophilic compounds is thus impaired in diseases that cause fat malabsorption.

The development of special dosage forms and the use of various absorption promoters for lipophilic compounds have been extensively studied. For example, Hansen et al. in WO92/10927 disclose the use of specific triglycerides in an enteral preparation for the treatment of lipid malabsorption. This reference specifically discloses the use of a purified lipid having a medium chain acyl moiety at the sn-1 and sn-3 positions and a long chain acyl moiety at the sn-2 position.

U.S. Pat. No. 4,753,963 to Jandacek et al. discloses a nutritional fat suitable for enteral and parenteral products. This patent claims a triglyceride having an N-octanoyl acyl radical or moiety at the sn-1 and sn-3 positions. The sn-2 acyl radical comprises saturated acyl groups selected from the group consisting of N-heptanoyl, N-octanoyl, N-nonanoyl, N-decanoyl, N-undecanoyl, lauroyl, myristoyl, palmitoyl, and stearoyl. This reference also discloses the use of these fats in enteral products comprising carbohydrates, a source of amino acids and optionally, components such as vitamins and minerals.

DeMichele et al. in U.S. Pat. No. 5,661,180 discloses a structured lipid containing a gamma-linolenic acid or a dihomogamma-linolenic acid moiety together with an n-3 fatty acid residue and a medium chain fatty acid moiety. The DeMichele structured lipid is disclosed as being well adapted to the treatment of disease or stress states. This reference also teaches the use of the specific structured lipid to modulate metabolic response associated with trauma and inflammatory disease states.

U.S. Pat. No. 4,871,768 to Bistrian et al. discloses a structured lipid comprising n-3 fatty acid moieties and medium chain fatty acid moieties. More specifically, this



patent discloses a synthetic triglyceride comprising a glycerol backbone having three fatty acids esterfied thereto wherein the fatty acids are selected from a first group consisting of n-3 fatty acids and a second group consisting of caprylic acid, capric acid and mixtures thereof. This patent discloses the structured lipids as a dietary supplement for enhancing resistance to infection while providing good nutrition and an excellent source of energy. This reference, like those above, is directed to the use of structured lipids for nutritional value.

International Publication. No. WO95/31110 to Lien et al. discloses a co-randomized fat composition for use in nutritional products for pre-term and low birth weight infants. This reference discloses the co-randomization of oils such as palmitic acid oil and an oleic acid oil to yield a mixture of triglycerides having a substantially different chemical profile than that of the starting materials. This reference discloses the use of these specific structured lipids in enteral or parenteral products for infants to approach the fatty acid profile of human milk.

European Patent Application No. 0 347 843 to Tsushima et al. discloses the improved absorption of vitamin E by the digestive system through the use of lecithin and a free unsaturated fatty acid. This reference discloses the free unsaturated fatty acid as preferably being oleic acid or linoleic acid. This reference teaches improved absorption of vitamin E through the combined administration of vitamin E with lecithin (phosphatidylcholine derived from egg yolk or soy beans) in combination with a free unsaturated fatty acid such as linoleic acid or oleic acid.

Kimura et al. in *Chem Pharm. Bull.*, 37(2) 439–441 (1989) entitled, "Enhancement of Oral Bioavailability of d-α-Tocopherol Acetate by Lecithin-Dispersed Aqueous Preparation Containing Medium-Chain Triglycerides in Rats" reports the use of vitamin E/lecithin-dispersed aqueous preparations which increase the lymphatic absorption of vitamin E. This reference also investigated the use of polysorbate 80-solubilized aqueous emulsions of vitamin E and its absorption through the intestinal mucosa. These investigators found that the administration of MCT significantly enhanced the absorption of lecithin-dispersed aqueous preparations of vitamin E by the gastrointestinal tract. It is well established that the absorption of MCT occurs mainly via the portal circulation and not via the lymphatic route. In contrast, vitamin E is transported predominantly via the lymphatic system. It appears from this reference that the mechanism of absorption of vitamin E does not resemble the intestinal transport of MCT.

Two publications by Fukui et al., *J. Pharmacobio-Dyn.*, 12, 80–86 (1989) and *J. Pharmacobio-Dyn.*, 12, 754–761 (1989) report the enhancing effect of MCT on intestinal absorption of vitamin E as does the Kimura et al. reference above. These two references also support the conclusion that MCT absorption and vitamin E absorption use unrelated pathways.

Chen et al. report in "Absorption of Tocopherol in Intestinal Lymph Fistula Rat: Effects of Triolein and Phosphatidylcholine" *Gastroenterology* 108:A720, (1995), that the absorption of vitamin E is influenced by the presence of triolein (triglyceride) and phosphatidylcholine (lecithin). The use of lecithin promotes the water miscibility of the vitamin E. When the same amount of fatty acid was infused in the form of triolein or phosphatidylcholine with vitamin E, the amount of lipid transported to the lymph was similar. In contrast, the transport of vitamin E into lymph was significantly reduced in the animals infused with phosphati-

dylcholine as compared to those infused with the triglyceride. It thus appears from this work that it is not possible to predict the level of absorption of vitamin E based on the efficiency of triglyceride absorption.

The inclusion of polyunsaturated fatty acids in bile salt micelles is reported to depress α-tocopherol absorption by the rat small intestine. See Muralidhara et al., "Intestinal Absorption of α-Tocopherol in the Unanesthetized Rat. The Influence of Luminal Constituents on the Absorptive Process." *J. Lab. Clin. Med.*, 90:85–91, (1977). It is known that polyunsaturated fatty acids are well absorbed by the gastrointestinal tract. However, Muralidhara et al. demonstrates that the absorption of vitamin E is suppressed by the presence of polyunsaturated fatty acids. The experiments indicate that micellar expansion with polyunsaturated fatty acids interferes with the absorption of tocopherol and may result in deficiency of the vitamin. This reference also supports the belief that better triglyceride absorption is not always associated with enhanced absorption of fat soluble vitamins.

MacMahon et al. have demonstrated in rats with bile diversion that a polar lipid such as oleic acid, is well absorbed into the lymphatic system from an emulsion (from bile salt micelles) while the non-polar α-tocopherol was poorly absorbed from the emulsion. See MacMahon et al. "Comparison of the Absorption of a Polar Lipid, Oleic Acid, and a Non-Polar Lipid, α-Tocopherol from Mixed Micellar Solutions and Emulsions", *European Journal of Clinical Investigation* 1:160–166, 1970. This publication also supports the position that good triglyceride absorption, from an emulsion, is not always associated with good vitamin absorption.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graphic representation of lymphatic retinol absorption for the non-injury model experiment conducted in Example 2.

FIG. 2 is a graphic representation of lymphatic tocopherol absorption for the non-injury model experiment conducted in Example 2.

FIG. 3 is a graphic representation of lymphatic retinol absorption for the ischemia/reperfusion injury model experiment conducted in Example 3.

FIG. 4 is the graphic representation of lymphatic tocopherol absorption for the ischemia/reperfusion injury model experiment conducted in Example 3.

FIG. 5 describes the study design with a flow diagram for Examples 2 and 3.

The results set forth in FIGS. 1–4 are statistically significant at p<0.01 (see *). A two way analysis of variance model was employed for each treatment, lipid type and their interaction effects in the model were used to compare the area under each curve. Models were extended to included contrast statements comparing the physical mix and the structured triglyceride within each treatment group.

## SUMMARY OF THE INVENTION

The present invention has many aspects. In one broad aspect, the invention relates to the discovery that, compared to conventional oils or their physical mixtures, structured glycerides containing at least 33 wt. % of acyl moieties having 4 to 12 carbon atoms, at least 30 wt. % of acyl moieties having more than 12 carbon atoms and an equivalent carbon number ("ECN") ranging from greater than 30 to less than 48 will greatly facilitate the absorption of lipophilic compounds such as fat soluble vitamins, nutrients and drugs. Thus, the invention provides a method for enhancing the absorption of lipophilic compounds in an animal, said method comprises administering to said animal:

a) at least one lipophilic compound in conjunction with

b) a structured glyceride component containing at least 33 wt. % acyl moieties having 4 to 12 carbon atoms, at least 30 wt. % of a acyl moieties having more than 12 carbon atoms and an equivalent carbon number (ECN) of greater than 30 to less than 48.

The structured glyceride component typically includes triglycerides. A structured triglyceride useful in this invention comprises 33 to 70 wt. % acyl moieties of medium chain length (i.e. 4 to 12 carbon atoms). More preferably the medium acyl chains comprise 45 to 70 wt. %, most preferably 50 to 65 wt. %. At all weight percents, the length of the medium acyl chains is preferably 4 to 12 carbon atoms, more preferably 6 to 12, most preferably 8 to 10 carbon atoms. The 30 to 67 wt. % remainder of the structured triglyceride is typically a long chain (13–22 carbon atoms) acyl moiety. More preferably the long acyl chains comprise 30 to 55 wt. %, most preferably 35 to 50 wt. %. Preferably, said long chain acyl moiety at all weight percents comprises a long chain polyunsaturated fatty acid residue. The structured glyceride component is preferably characterized as comprising at least 40% of a species with equivalent carbon number (ECN) of greater than 30 to less than 48, more, preferably ECN of about 32 to about 42.

The lipophilic compounds of this invention may be selected from oil soluble drugs, nutrients and vitamins.

The invention further provides a composition suitable for administration to an animal comprising:

a) at least one lipophilic compound and

b) a structured glyceride component containing at least 33 wt. % acyl moieties having 4 to 12 carbon atoms, at least 30 wt. % of a acyl moieties having more than 12 carbon atoms and an equivalent carbon number (ECN) of greater than 30 to less than 48.

In a further aspect, the improved method is useful in an animal that suffers from lipid malabsorption. In such a case, the composition preferably comprises at least one lipophilic compound and a structured glyceride component, said structured glyceride component comprising at least 33 wt. % acyl moieties of 4 to 12 carbon atoms; at least 30 wt. % long chain polyunsaturated acyl moieties and an equivalent carbon number (ECN) of greater than 30 to less than 48.

Other aspects of the invention are described throughout the application.

## DETAILED DESCRIPTION

### General Terminology

According to this invention, lipophilic compounds are used in conjunction with a structured glyceride component. By "in conjunction with" we mean that the lipophilic compounds are administered to said animal within one hour of administration of the structured glyceride component. More preferably, the lipophilic compounds are administered at the same time as the structured glyceride component, most preferably admixed in the same composition, such as enteral nutritionals, nutritional supplements, tablets, pills, capsules, suppositories, sprays, lozenges, drops, lotions, ointments, microcapsules and liposomes.

The term "lipid" generally denotes a heterogeneous group of substances associated with living systems which have the common property of being insoluble in water, can be extracted from cells by organic solvents of low polarity such as chloroform and ether. The terms "lipophilic compound"

and "lipid soluble compound" thus refers to those compounds that have greater solubility in organic solvents such as ethanol, methanol, ethyl ether, acetone, chloroform and benzene and fats and oils than in water. Specific compound solubility is listed in references such as Section C of the CRC Handbook of Chemistry and Physics, 67th Edition, CRC Press and the Merck Index. Lipid soluble compounds include drugs, hormones, vitamins, nutrients and other selected lipophillic compounds, as described in detail later.

The term "structured lipid" generally refers to an oil or fat that contains specific fatty acyl residues in a specific position on the glycerol backbone. As used in this invention, a "structured glyceride component" refers to a glyceride mixture characterized in that it may contain mono-, di- and triglycerides, more typically di- and triglycerides, ideally a higher percentage of triglycerides. At least 40%, of the triglyceride species have about 33 to 70 wt. % of acyl moieties having 4 to 12 carbon atoms; about 30 to 67 wt. % of acyl moieties having more than 12 carbon atoms and an equivalent carbon number of greater than 30 to less than 48.

A glyceride is an ester of glycerol (1,2,3-propanetriol) with acyl radicals of fatty acids and is also known as an acylglycerol. If only one position of the glycerol molecule is esterified with a fatty acid, a "monoglyceride" is produced; if two positions are esterified, a "diglyceride" is produced; and if all three positions of the glycerol are esterified with fatty acid a "triglyceride" or "triacylglycerol" is produced. A glyceride is called "simple" if all esterified positions contain the same fatty acid; or "mixed" if different fatty acids are involved. The carbons of the glycerol backbone are designated sn-1, sn-2 and sn-3, with sn-2 being in the middle and sn-1 and sn-3 being the ends of the glycerol.

Naturally occurring oils and fats consist largely of triglycerides wherein the 3 fatty acyl residues may or may not be identical. The term "long chain triglycerides (LCT)" means both a simple and mixed triglyceride containing fatty acids with more than 12 carbon atoms (long chain fatty acids—"LCFA"), whereas the term "medium chain triglycerides (MCT)" means both a simple and mixed triglyceride containing fatty acids with 4 to 12 carbon atoms.

The term "ECN" or "equivalent carbon number" means the sum of the number of carbon atoms in the acyl chains of a glyceride molecule. For example, tripalmitin (tripalmitic glycerol), which is a simple triglyceride containing 3 acyl radicals of 16 carbon atoms, has an ECN of 3×16=48. Conversely, a triglyceride with an ECN=40 may have "mixed" acyl chain lengths of 8, 16 and 16; 10, 14 and 16; 8, 14 and 18, etc. Naturally occurring oils are frequently "mixed" with respect to specific fatty acids, but tend not to contain LCFAs and MCFAs on the same glycerol backbone. Thus, triacylglycerols with ECN's of 24–30 typically contain predominately medium chain fatty acids; while triacylglycerols with ECN's of greater than 43 typically contain predominantly long chain fatty acids. Triacylglycerols having an ECN's of 3242 typically contain one or two MCFA in combination with one or two LCFA's to "fill" the triglyceride. Triacylglycerols with ECN's in the range of greater than 30 to less than 48 typically represent mixed triacylglycerol species that are essentially unique to the structured triglyceride and are absent from or are present in significantly lower concentrations in physical mixtures.

Many of the properties of food lipids can be accounted for directly in terms of their component fatty acids. The fatty acids that occur in foodstuffs usually contain an even number of carbon atoms in an unbranched chain, e.g., lauric or dodecanoic acid. Besides the saturated fatty acids, of which lauric acid is an example, fatty acids may have 1, 2 or sometimes up to 6 double bonds and are, therefore, unsaturated. The number and position of double bonds in fatty acids are designated by a convention of nomenclature typically understood by the organic chemist. For example, arachidonic acid ("AA" or "ARA") has a chain length of 20 carbons and 4 double bonds beginning at the sixth carbon from the methyl end. As a result, it is referred to as "20-4n-6". Similarly, docosahexaenoic acid ("DHA") has a chain length of 22 carbons with 6 double bonds beginning with the third carbon from the methyl end and is thus designated "22:6n-3".

The terms "wt. %" or "weight percent" means the ratio of the mass of the recited component to the mass of the specified ingredient or entire composition multiplied by 100. For example, "a triglyceride comprising 40 wt. % acyl moieties of 10 carbon atoms" means that 100 gms of the triglyceride oil consists of 40 gms of 10 carbon atoms acyl radicals and 60 gms of other components, including other acyl radicals and the glycerol backbone.

The term "fish oil" means the oil derived from fish sources, such as menhaden, sardine, cod and the like. Fish oil has gained much attention in recent years as Eskimos, who consume high levels of fish oils, have a remarkably low incidence of arterial disease. Fish oils are rich in polyunsaturated long chain fatty acids such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3).

Compositions

The compositions useful in the method of enhancing the absorption and transport of lipophilic compounds comprise a structured glyceride component of at least 33 wt. % randomly esterified MCFA. The remainder of the fatty acid moieties are typically LCFA. The source of the MCT and LCT to prepare the structured glyceride component is not critical. Typical sources of MCT such as fractionated coconut oil and fractionated palm kernel oils are known to those skilled in the art. Sources of LCFA include the oils derived from borage, black currant seed, corn, coconut, canola, soybean, marine oils, fungal oils, safflower, high oleic safflower, sunflower, high oleic sunflower, olive, evening primrose, cottonseed, rice bran, grapeseed, flaxseed, butterfat, garlic, peanuts, almonds, walnuts, wheat germ, egg, sesame, lard, tallow and mutton.

In a more preferred embodiment, the structured glyceride of the invention also contains a long chain polyunsaturated fatty-acid (hereinafter "LCPUFA") such as the n-6, n-9 and/or n-3 long chain fatty acids. Known sources of LCPUFA include fish or marine oil, egg yolk lipid, single cell oils (e.g., algal oils and fungal oils), it being understood in the art that some sources are better than others for achieving higher amounts of a specific LCPUFA. Other edible, semi-purified or purified sources of LCPUFA will be evident to persons skilled in the art. For example, new sources of LCPUAs may be developed through the genetic manipulation of vegetables and oil bearing plants. The use of such recombinant oils are also contemplated in the present invention.

The structured glycerides useful in the present invention contain both MCFA and LCFA. The structured tryglycerides useful in this invention are chemically distinct and offer unique advantages from the starting materials from which they are derived. One aspect of the present invention resides in the discovery that structured triglycerides that contain a certain mixture of MCFA and LCFA are subject to rapid hydrolysis and absorption in comparison to LCT's. In addition, the structured triglycerides of this invention are primarily absorbed and transported through the lymphatic system as opposed to the hepatic route.

In native fats and oils, the various fatty acids are esterified through one of the three hydroxy groups of the glycerol molecule in an ordered pattern that is characteristic of the particular fat or oil. In general, the naturally occurring, long chain, saturated fatty acids (e.g., $C_{16}$–$C_{18}$) are predominantly at the sn-1 and sn-3 positions, while the mono- and polyunsaturated fatty acids are at the sn-2 or middle position of the triglyceride molecule. There are only a small number of naturally-occurring "simple triglycerides", for example, tripalmitin ($C_{48}$), triolein ($C_{18}$) and the like.

The structured glyceride component of this invention will predominantly contain triglycerides, 50% by weight or more, frequently about 90% by weight. Of these triglycerides (whatever their proportion) at least 40% by weight have an ECN greater than 30 and less than 48. More preferably, the structured glyceride component will contain at least 60% by weight the ECN greater than 30 and less than 48 species, most preferably at least 60% by weight the ECN of about 32 to about 42 species.

Utility

A further aspect of the present invention resides in the discovery that a structured glyceride according to the invention can act as a carrier to facilitate the delivery (absorption) of fat soluble vitamins (e.g. A, E, D, K and carotenes) as well as other lipophilic natural and synthetic drugs. While there may exist some evidence that the use of MCT or LCT oils alone may enhance the absorption of tocopherol, the state of the art is at best unable to predict with any level of certainty which oils and which compounds will evidence enhanced lymphatic absorption. There is no suggestion or disclosure in the prior art that a particular structured triglyceride would enhance the lymphatic absorption and transport of lipophilic compounds in healthy animals or in animals with lipid malabsorption. Further, the prior art teaches that the absorption mechanisms of fat soluble vitamins do not resemble the absorption of dietary triglycerides. Thus, this invention is based, in part, on the discovery that a certain class of structured glyceride will significantly improve the absorption of the lipophilic compound into the lymphatic system. This invention is an especially meaningful discovery since patients with malabsorption diseases such as Crohn's disease are at increased risk of developing vitamin E, A and/or D deficiencies and these patients also present special problems in the delivery of lipophilic drugs.

The lipophilic compounds of this invention may be selected from oil soluble drugs, nutrients and vitamins. Representative of the oil soluble drugs useful in the present invention are the natural and synthetic forms of immunosuppressive agents such as Cyclosporin™, protease inhibitors such as Ritonavir™, macrolide antibiotics and oil soluble anesthetics such as Propofol™ are also useful in this invention. The synthetic and natural forms of steroidal hormones such as estrogens, estradiols, progesterone, testosterone, cortisone, phytoestrogens, dehydroepiandrosterone (DHEA), growth hormones and the like can also be used in this invention. Also useful in the present invention are the oil soluble acids and alcohols such as tartaric acid, lactylic acid, butylated hydroxyanisole, butylated hydroxytoluene, lignin, sterols, polyphenolic compounds, oryzanol, cholesterol, lignin, phytosterols, flavonoids such as quercetin and reservatol, diallyl disulfides and the like. Polar lipids such as the phospholipids and ether lipids can also be used. Oil soluble vitamins including the synthetic and natural forms of vitamin A, E, D and K, carotenoids and lycopenes can also be used in this invention.

The present invention also contemplates the use of the structured glycerides in a nutritionally complete food product or a nutritional supplement containing a lipophilic compound whose absorption is desired to be enhanced. The food product or supplement may comprise the fat composition of the invention, additional oils, an amino nitrogen source such as protein, protein hydrolysates or elemental amino acids, a carbohydrate source and appropriate levels of the oil soluble vitamins, nutrients and/or drugs. The product may be in a ready-to-feed liquid form, or in the form of a powder or concentrated liquid adapted to provide a ready-to-feed product by the addition of water and stirring.

In addition to nutritional formulation, the composition according to the invention may be formulated as a tablet, pill, capsule, suppository, spray, lozenge, ear drops, eye drops or topical formula for application to the skin (i.e. lotions, ointments, trans-dermal patches and the like). The structured glyceride component and lipophilic compound(s) may also be microencapsulated or in the form of liposomes.

The lipophilic compounds useful in the present invention can be at concentrations from a few parts per million to 90% by wt, including any interim concentrations, as is appropriate for delivering the particular lipophilic compound. When in the form of a dietary supplement or pharmaceutical preparation, the structured glyceride and lipophilic compound may comprise from 10–100 wt. % of the dietary supplement or pharmaceutical preparation. An enteral formula utilizing the present invention will typically contain from 1–20% by wt. of the structured glyceride component/lipophilic compound. Those skilled in the art of preparation of enteral formulas will be readily able to determine what sources of amino nitrogen, carbohydrates, vitamins and minerals would be suitable for combination with the structured triglyceride/lipophilic compound physical mixture of the present invention.

Process of Making

The structured glycerides of this invention may be prepared by any procedure commonly used to make structured lipids. For example, an interesterification or transesterification reaction made by mixing oils, or selective fractions of the oils, in stoichiometric proportions and then causing the transesterification reaction to proceed using catalysis or enzymes could be used. In addition, one skilled in the art could genetically engineer the oil bearing plants to produce the specific structured glycerides described in this invention. Although a standard transesterification procedure may result in a component mixture containing the structured glycerides of the invention along with other oils, such a component mixture is intended to be included within the claims.

It is possible to source MCT oils as starting materials to prepare the structured lipids useful in this invention. MCT oils, such as fractionated coconut oil and fractionated palm kernel oils, are obtained by the hydrolysis of coconut and palm kernel oils and the distillation of the fatty acids. The fatty acids are then re-esterified to the glycerol molecules to obtain the MCT oil.

The chemical interesterification process used for the preparation of the structured triglycerides in the following examples is according to the teachings found in the "Oils and Fats Manual, A Comprehensive Treatise", Vol. 2, Chapter 11, *Transformation of Fat for Use in Food Products*, pgs. 923–925, the entire teaching of which is hereby incorporated by reference. Chemical interesterification, also called co-randomization (since it alters the non-random distribution of nature) may be accomplished by heating a mixture of oils for a short period of time (e.g. from 0.5 to 4 hours, preferably 0.5 to 2 hours at temperatures of 100–140° C., preferably 110–130° C.) in the presence of a catalyst such as sodium methylate or sodium methoxide (e.g. range from

0.05 to 0.5% by wt., more preferably from 0.1 to 0.3% by wt.) The fatty acids leave their natural position on the triglyceride and rearrange in a random fashion (presumably equally on each of the three positions). Thus, about one third of each individual fatty acid will re-esterify at the sn-1 position, about one third on sn-2 and about one third on sn-3.

The examples below describe the distribution resulting from the co-randomization of equal weight parts of an MCT oil (having medium chain fatty acids, "MCFA" or M) and a fish oil (having long chain polyunsaturated fatty acids, "LCPUFA" or L) in addition to the distribution resulting from the co-randomization with twice as much MCFA as LCPUFA. Of course, other ratios of MCFA to LCPUFA are within the invention, including from about 1:3 to about 3:1, more typically from about 1:2 to about 2:1. The distribution of triglyceride entities that result from these combinatorial probabilities can be represented as follows with the approximate yields shown:

TABLE 1

STRUCTURED TRIGLYCERIDE PROBABILITY OF OCCURRENCE

| Triglyceride | Probability of Occurrence 1:1 MCFA and LCPUFA mix | | Triglyceride | Probability of Occurrence 2:1 MCFA and LCPUFA mix | |
|---|---|---|---|---|---|
| MLM | $(1/2)^2 = 1/8$ | (12.5%) | MLM | $(2/3)^2(1/3) = 4/27$ | (14.8%) |
| LMM | $(1/2)^2 = 1/8$ | (12.5%) | LMM | $(1/3)(2/3)^2 = 4/27$ | (14.8%) |
| MML | $(1/2)^2 = 1/8$ | (12.5%) | MML | $(2/3)^2(1/3) = 4/27$ | (14.8%) |
| Total 2 MCFA, 1 LCPUFA yield | | 37.5% | Total 2 MCFA, 1 LCPUFA yield | | 44.4% |
| LML | $(1/2)^2 = 1/8$ | (12.5%) | LML | $(1/3)^2(2/3) = 2/27$ | (7.4%) |
| LLM | $(1/2)^2 = 1/8$ | (12.5%) | LLM | $(1/3)^2(2/3) = 2/27$ | (7.4%) |
| MLL | $(1/2)^2 = 1/8$ | (12.5%) | MLL | $(2/3)(1/3)^2 = 2/27$ | (7.4%) |
| Total 1 MCFA, 2 LCPUFA yield | | 37.5% | Total 1 MCFA, 2 LCPUFA yield | | 22.2% |
| Subtotal | | 75% | Subtotal | | 66.6% |
| LLL | $(1/2)^3 = 1/8$ | 12.5% | LLL | $(1/3)^3 = 1/27$ | 3.7% |
| MMM | $(1/2)^3 = 1/8$ | 12.5% | MMM | $(2/3)^3 = 8/27$ | 29.6% |
| Total | | 100% | Total | | 99.9% |

New, non-natural triglycerides are created in the random re-esterification of the MCFA and the LCPUFA as shown in Table 1. An exemplary co-randomized structured triglyceride made according to this process is described in Example 1, below. As described above, altering the ratio of MCT oil (MCFA) to fish oil (LCFA) will alter the yield percentages in mathematically predictable ways. The formula for the probability of occurrence of a specific triglyceride is P=(3, n)(pl")(pm$^{(3-n)}$) where pl is the probability of occurrence of L, pm is the probability of occurrence of M, n is the number of Ls in the triglyceride and (3,n) is the total number of ways n objects can be selected from 3 objects. For any triglyceride the combinatorial expression (3,n) resolves to one of the following: (3,1)=3, (3,2)=3 and (3,3)=1. Therefore, the probability of occurrence of a triglyceride twice as much MCFA as LCPUFA is (3,1)(1/3)$^1$(2/3)$^{3-1}$=12/27 or 44.4%, the sum of the probabilities of occurrence of the entities: MML, MLM, LMM (see Table 1).

An important difference between the structured triglyceride component and a physical mix of its constituent oils is found in the molecular species of the triglycerides. The individual molecular species of the structured glyceride component are designated by the Equivalent Carbon Number (ECN). The interesterification (or co-randomization) of the constituent oils creates new triglyceride species which are unique and are absent in the constituent oils. Specifically, the described co-randomization process produces triglyceride species having ECN about 32 to 42 that are simply not found in physical mixtures of the constituent oils. Structured glyceride components of this invention will preferably contain at least 40% by weight of ECN greater than 30 to less than 48 species. More preferably, the structured glyceride component will contain at least 60% by weight of the ECN greater than 30 to less than 48, most preferably at least 60% by weight of the ECN of about 32 to about 42 species.

Without being bound to any theory or mechanism, the inventors believe that these differences, in part, are responsible for the structured lipid's ability to enhance the absorption and transport of lipophilic compounds into the lymphatic system as compared to the physical blend of oils.

The following Examples are intended to illustrate the present invention, not limit it. Rather, the invention is defined by the appended claims.

## EXAMPLE 1

Part A. Physical mixture: Equal weights of MCT oil (Stepan, Inc. of New Jersey, USA) and fish oil (Mochida, Ltd. of Tokyo, Japan) were blended and mixed well to comprise the "physical mix" experimental oil.

Part B. Structured Triglyceride: Equal weights of MCT oil (Stepan, Inc. of New Jersey, USA) and fish oil (Mochida, Ltd. of Tokyo, Japan) were co-randomized according to the teachings found in "Oils and Fats Manual, A Comprehensive Treatise", Vol 2, Chapter 11, *Transformation of Fat for Use in Food Products*, pgs. 923–925 using sodium methoxide as the catalyst, to manufacture the "structured triglyceride component" experimental oil.

The fatty acid composition of the structured triglyceride component of Part B and the physical mixture of Part A is set forth in Table 2. As seen in Table 2, below, the fatty acid compositions of the two experimental oils are essentially identical.

TABLE 2

FATTY ACID COMPOSITION OF EXPERIMENTAL OILS

| Fatty Acid | MCT Oil Weight % | Fish Oil Weight % | Physical Mix Weight % | Structured Triglyceride Weight % |
|---|---|---|---|---|
| 8:0 | 55.7 | — | 27.6 | 27.0 |
| 10:0 | 43.4 | — | 20.7 | 20.5 |
| 12:0 | 0.8 | 0.3 | 0.5 | 0.5 |
| 14:0 | 0.1 | 5.9 | 3.0 | 2.9 |
| 16:0 | — | 9.5 | 4.9 | 4.7 |
| 16:1n-7 | — | 8.3 | 4.2 | 4.1 |
| 18:0 | — | 1.2 | 0.6 | 0.6 |
| 18:1n-9 | — | 11.7 | 6.0 | 5.7 |
| 18:2n-6 | — | 1.7 | 0.9 | 0.8 |
| 18:4u-3 | — | 2.8 | 1.5 | 1.5 |

TABLE 2-continued

FATTY ACID COMPOSITION OF EXPERIMENTAL OILS

| Fatty Acid | MCT Oil Weight % | Fish Oil Weight % | Physical Mix Weight % | Structured Triglyceride Weight % |
|---|---|---|---|---|
| 20:1n-9 | — | 1.9 | 1.0 | 1.0 |
| 20:4n-6 | — | 2.8 | 1.5 | 1.5 |
| 20:5n-3 | — | 28.7 | 14.7 | 15.1 |
| 22:5n-3 | — | 3.2 | 1.7 | 1.8 |
| 22:6n-3 | — | 13.1 | 6.8 | 8.0 |
| Others | — | 8.5 | 4.4 | 4.3 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 |

Table 3 set forth the ECN species profile of the two experimental oils of parts A and B, above.

TABLE 3

TRIGLYCERIDE PROFILE OF EXPERIMENTAL OILS

| Sample ID ECN* | Physical Mix % Weight | Structured Triglycerides % Weight |
|---|---|---|
| 24 | 11.5 | 4.3 |
| 26 | 21.8 | 8.3 |
| 28 | 14.4 | 5.7 |
| 30 | 3.4 | 2.8 |
| 32 | 0.0 | 5.9 |
| 34 | 0.0 | 8.8 |
| 35 | 0.0 | 10.8 |
| 37 | 0.0 | 11.2 |
| 38 | 0.0 | 5.4 |
| 39 | 0.0 | 2.4 |
| 41 | 0.0 | 5.2 |
| 42 | 0.0 | 6.0 |
| 44 | 0.5 | 6.0 |
| 46 | 1.8 | 5.1 |
| 47 | 0.0 | 4.0 |
| 48 | 4.1 | 0.0 |
| 49 | 0.0 | 1.6 |
| 50 | 7.3 | 1.3 |
| 52 | 8.7 | 1.5 |
| 53 | 9.0 | 1.3 |
| 55 | 7.7 | 1.1 |
| 57 | 5.4 | 0.7 |
| 58 | 2.4 | 0.0 |
| 59 | 0.4 | 0.8 |
| 60 | 1.7 | 0.0 |
| Total | 100.0 | 100.0 |
| Sum - ECN 32-47 | 0 | 70.8 |

*ECN: Equivalent carbon number.

It can be seen that the species of ECN 32 to 47 are absent or nearly absent in the physical mix oil; yet these species comprise 70.8% by weight of the structured triglyceride oil. Furthermore, greater than half (55.7%) of the triglyceride oil are species of ECN 32 to 42 and are these are completely nonexistent in the physical mixture. The co-randomization process has clearly created new chemical species.

## EXAMPLE 2

This experiment was conducted to determine if a structured glyceride consisting of a co-randomized MCT/fish oil would enhance the absorption of lipophilic compounds such as vitamin E (tocopherol) and retinol (vitamin A) using a lymph fistula rat model based upon a procedure described by Fujimoto et al., "Effect of Ischemia-Reperfusion on Lipid Digestion and Absorption in Rat Intestine", *Am. J. Physiol.*, 260: G595–G602 (1991), the entire teaching of which is hereby incorporated by reference. This model was designed to represent fat absorption mode of the healthy intestinal tract (i.e., not compromised through disease or ischemic event). The lymph fistula rat model is extremely accurate in measuring and quantitating lipid absorption.

The physical mix (Part A) and structured triglyceride component (Part B) of Example 1 were used. Radiolabeled retinol and α-tocopherol used in this example and in Example 3 are commercially available from Hoffman-LaRoche, Inc. (New Jersey, USA).

Male Sprague Dawley rats weighing between 280 and 330 grams were all fed a normal Purina Rat Chow for one week. Rats were fasted overnight prior to surgery and under anesthesis, a laparotomy was performed, the intestinal lymph duct was cannulated according to the procedure of Tso et al., "The Absorption of Lipid and Lipoprotein Synthesis", *Lipid Research Methodology*, Chapter 5: 191–216 (1984) Alan R. Liss, Inc., N.Y., N.Y, the entire teaching of which is hereby incorporated by reference. The superior mesenteric artery was isolated but not occluded as in the injury model used in Example 3. A silicon infusion tube (1.6 mm OD) was placed in the stomach for infusion of saline, structured lipid/lipophilic compound or emulsified physical mix/lipophilic compound. The fundic incision was closed by a purse string suture. The animals were allowed to recover for 24 hours before experimental oil/lipophilic compound infusion began.

Animals were randomly assigned to two groups in this non-injury model. Animals were intragasirically infused 24 hours after surgery with 1.0 ml of MCT/fish oil structured glyceride (Example 1, Part B) for Group B or 1.0 ml of its equivalent physical mixture (Example 1, Part A) for Group A. Radiolabeled tocopherol and retinol (lipophilic compounds) were added to the structured triglyceride component and to the physical mix. 90 mM of tocopherol was administered to each animal in combination with 1 mCi of $^{14}$C-tocopherol. 0.528 mM of retinol was administered to each animal in combination with 10 mCi of $^{3}$H-retinol.

Lymph was collected in pre-cooled tubes beginning hourly for eight (8) hours after initiation of lipid infusion. At the end of the lipid infusion, the animals were sacrificed by exsanguination.

Radioactivity was measured in an aqueous miscible scintillant (Poly-Fluor, Packard, Downers Grove, Ill.). Samples were counted for 10 minutes in a liquid scintillation spectrophotometer (LKB Model. 1209, Pharmacia, Inc.). Samples were corrected for quenching by reference to a series of $^{14}$C and $^{3}$H standards that were progressively quenched.

Lymph lipids were extracted and the methyl ester derivatives of the fatty acids were analyzed using a Hewlett Packard Gas Chromatograph, Model 5890A with a capillary column packed with 10% SP-2330 on 80/100 Supelcoport (Supelco, Inc., Bellefonte, Pa.).

The lymphatic retinol absorption measured in pmol per hour is presented in FIG. 1. FIG. 1 demonstrates that the structured triglyceride component according to this invention increased the lymphatic absorption of retinol over the entire 8 hour study period. In similar fashion, FIG. 2 demonstrates that the structured glyceride component according to this invention enhances the lymphatic absorption of tocopherol over the entire 8 hour study period. This experiment clearly demonstrates that the use of a structured triglyceride, in accordance with the invention, in combination with a lipophilic compound, such as vitamins A and E, can result in at least a 30% higher ($p<0.01$) lymph output of

the lipophilic compound compared to the corresponding physical mix in this normal absorption model. It is important to note that the lymph tocopherol and retinol increased rapidly and maintained a significantly higher output ($p<0.01$) with the structured glyceride component versus the physical mix.

EXAMPLE 3

Ischemia/Reperfusion Injury Model

This experiment was conducted in a manner similar to Example 2 except that the ischemic/reperfusion injury model was used. This model was used to simulate the lipid malabsorption conditions associated with diseases such as Short Bowel Syndrome, Crohn's disease and the like. Animals were divided into two groups and given the physical mix (Group A) or the structured triglyceride component (Group B) of Example 2. The major difference in this experiment was that the superior mesenteric artery was occluded for 25 minutes with a clamp and at the end of that ischemic period the clamp was released with a few drops of lidocaine which was applied directly onto the artery to facilitate reperfusion.

FIG. 3 graphically represents the lymphatic retinol absorption over the 8 hour study period. The data represents that over the initial 3 hour period, the lymphatic absorption of retinol was about equal between the structured triglyceride component and the physical mixture.

However, after about 3 hours, the structured triglyceride component evidences an enhancement in the retinol absorption over the physical mix. At the end of the 8 hour study, the structured triglyceride component was providing more than twice the amount of retinol to the lymphatic circulation than the physical mixture.

FIG. 4 represents the data collected regarding the absorption of tocopherol. In similar fashion, the initial 2 hour period of tocopherol absorption was essentially equal between the structured triglyceride component and the physical mix, however, after about 2 hours, the structured triglyceride showed a significantly enhanced level of lymphatic tocopherol absorption. This experiment clearly demonstrates that the structured triglyceride component can result in at least a 30% ($p<0.01$) higher lymph output of the lipophilic compound to the corresponding physical mix in the malabsorptive rat model.

Industrial Applicability

The medical community continues to seek methods to overcome the problems associated with the administration of oil soluble drugs, nutrients and vitamins. The need to provide adequate absorption of these lipophilic compounds to patients with malabsorptive diseases such as Crohn's disease or Short bowel disease, present special problems. The novel method of this invention which comprises the administration of a structured glyceride in conjunction with a lipophilic compound such as vitamins A and E, oil soluble drugs and nutrients, fulfills this long felt need. The method of the present invention can be accomplished through the administration of pills, capsules, suppositories, lozenges, transdermal patches, sprays, drops, dietary supplements, pharmaceutical preparations and the like that utilize the structured glyceridcnipophilic compound physical mixture described herein.

Modifications and alternative embodiments of the invention will be apparent to those skilled in the art in view of the foregoing description. Accordingly, this description is to be construed as illustrative only and is for the purpose of teaching those of skill in the art the manner of carrying it out.

We claim:

1. A method for enhancing an animal's absorption of at least one lipophilic compound, said method comprising administering orally to said animal:

(a) at least one lipophilic compound in conjunction with

(b) a structured glyceride component characterized in that it contains some triglyceride species and at least 40% of the triglyceride species have:

(i) about 33 to 70 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms;

(ii) about 30 to 67 wt. % of long chain acyl moieties having more than 12 carbon atoms; and

(iii) an equivalent carbon number of greater than 30 to less than 48.

2. The method according to claim 1 wherein said structured glyceride component predominantly comprises triglycerides.

3. The method according to claim 2 wherein said triglycerides comprise 45 to 65 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms.

4. The method according to claim 2 wherein said triglycerides comprise 35 to 55 wt. % of long chain acyl moieties having more than 12 carbon atoms.

5. The method according to claim 2 wherein said triglycerides comprise 45 to 65 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms and 35 to 55 wt. % of long chain acyl moieties having more than 12 carbon atoms.

6. A method according to claim 5 in which said at least 60% by weight of said structured glyceride has an equivalent carbon number of about 32 to about 42.

7. A method according to claim 2 in which said structured glyceride has an equivalent carbon number of about 32 to about 42.

8. The method according to claim 1 wherein said lipophilic compound is selected from the group consisting of oil soluble drugs, nutrients and vitamins.

9. The method according to claim 8 wherein said lipophilic compound is a vitamin selected from the group consisting of vitamin A and vitamin E.

10. A method according to claim 8 in which said oil soluble drug is selected from the group consisting of immunosuppressive agents, protease inhibitors, macrolide antibiotics, anesthetics, estrogens, estradiols, progesterone, testosterone, cortisone, phytoestrogens, dehydroepiandrosterone, and growth hormone.

11. A method according to claim 8 in which said vitamins are selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, carotenoids, and lycopenes.

12. The method according to claim 1 wherein the structured glyceride component is prepared by interesterification of an oil containing predominantly medium chain triglycerides and an oil containing predominantly long chain triglycerides in a ratio from about 1:2 to about 2:1.

13. The method of claim 12 wherein said structured glyceride component is prepared by chemical interesterification.

14. A method according to claim 12 in which said lipophilic compound is an oil soluble drug is selected from the group consisting of immunosuppressive agents, protease inhibitors, macrolide antibiotics, anesthetics, estrogens, estradiols, progesterone, testosterone, cortisone, phytoestrogens, dehydroepiandrosterone, and growth hormone.

15. A method according to claim 12 in which said lipophilic compound is a vitamin selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, carotenoids, and lycopenes.

16. A method according to claim 12 in which said medium chain triglycerides are obtain from fractionated coconut oil, fractionated palm kernel oil, or a mixture thereof.

17. A method according to claim 12 in which said long chain triglycerides are obtained from an oil selected from the

group consisting of borage, black currant seeds corn, coconut, canola, soybean, marine oils, fungal oils, safflower, high oleic safflower, sunflower, high oleic sunflower, olive, evening primrose, cottonseed, rice bran, grapeseed, flaxseed, butterfat, garlic, peanuts, almonds, walnuts, wheat germ, egg, sesame, lard, tallow and mutton.

**18**. The method according to claim **1** wherein at least 40% of the triglyceride species have an equivalent carbon number of about 32 to about 42.

**19**. The method according to claim **1** wherein said animal suffers from a lipid malabsorption condition.

**20**. A method according to claim **1** in which said medium chain acyl moieties have from 6 to 12 carbon atoms.

**21**. A method according to claim **1** in which said medium chain acyl moieties have from 8 to 10 carbon atoms.

**22**. A method according to claim **1** in which said structured glyceride has an equivalent carbon number of about 32 to about 42.

**23**. A method according to claim **1** in which said structured glyceride is produced using at least one recombinant oil.

**24**. A composition suitable for oral administration comprising:

(a) at least one lipophilic compound; and

(b) a structured glyceride component characterized in that it contains some triglyceride species and at least 40% of the triglyceride species have:

(i) about 33 to 70 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms;

(ii) about 30 to 67 wt. % of long chain acyl moieties having more than 12 carbon atoms; and

(iii) an equivalent carbon number of greater than 30 to less than 48.

**25**. The composition according to claim **24** wherein said structured glyceride component predominantly comprises triglycerides.

**26**. The composition according to claim **25** wherein said triglycerides comprise 45 to 65 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms.

**27**. The composition according to claim **25** wherein said triglycerides comprise 35 to 55 wt. % of long chain acyl moieties having more than 12 carbon atoms.

**28**. The composition according to claim **25** wherein said triglycerides comprise 45 to 65 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms and 35 to 55 wt. % of long chain acyl moieties having more than 12 carbon atoms.

**29**. A composition according to claim **28** in which said at least 60% by weight of said structured glyceride has an equivalent carbon number of about 32 to about 42.

**30**. A composition according to claim **25** in which said structured glyceride has an equivalent carbon number of about 32 to about 42.

**31**. The composition according to claim **24** wherein the structured glyceride component is prepared by interesterification of an oil containing predominantly medium chain triglycerides and an oil containing predominantly long chain triglycerides in a ratio from about 1:2 to about 2:1.

**32**. The composition according to claim **31** wherein said structured glyceride is prepared by chemical interesterification;

**33**. A composition according to claim **32** which said structured glyceride is produced using at least one recombinant oil.

**34**. A composition according to claim **31** in which said lipophilic compound is an oil soluble drug is selected from the group consisting of immunosuppressive agents, protease inhibitors, macrolide antibiotics, anesthetics, estrogens, estradiols, progesterone, testosterone, cortisone, phytoestrogens, dehydroepiandrosterone, and growth hormone.

**35**. A composition according to claim **31** in which said lipophilic compound is a vitamin selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, carotenoids, and lycopenes.

**36**. A composition according to claim **31** in which said medium chain triglycerides are obtain from fractionated coconut oil, fractionated palm kernel oil, or a mixture thereof.

**37**. A composition according to claim **31** in which said long chain triglycerides are obtained from an oil selected from the group consisting of borage, black currant seed, corn, coconut, canola, soybean, marine oils, fish oils, fungal oils, safflower, high oleic safflower, sunflower, high oleic sunflower, olive, evening primrose, cottonseed rice bran, grapeseed, flaxseed, butterfat, garlic, peanuts, almonds, walnuts, wheat germ, egg, sesame, lard, tallow and mutton.

**38**. A composition according to claim **31** in which said long chain triglycerides are obtained from fish oil.

**39**. A composition according to claim **38** in which said medium chain triglycerides are obtained from fractionated coconut oil.

**40**. The composition according to claim **24** wherein said lipophilic compound is selected from the group consisting of oil soluble drugs, nutrients and vitamins.

**41**. The composition according to claim **40** wherein said lipophilic compound is a vitamin selected from the group consisting of vitamin A and vitamin E.

**42**. The composition according to claim **24** wherein at least 40% of the triglyceride species have an equivalent carbon number of about 32 to about 42.

**43**. A composition according to claim **24** in which said lipophilic compounds is an oil soluble drug is selected from the group consisting of immunosuppressive agents, protease inhibitors, macrolide antibiotics, anesthetics, estrogens, estradiols, progesterone, testosterone, cortisone, phytoestrogens, dehydroepiandrosterone, and growth hormone.

**44**. A composition according to claim **24** in which said lipophilic compounds is a vitamin selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, carotenoids, and lycopenes.

**45**. A composition according to claim **24** in which said medium chain acyl moieties have from 6 to 12 carbon atoms.

**46**. A composition according to claim **24** in which said medium chain acyl moieties have from 8 to 10 carbon atoms.

**47**. A composition according to claim **24** in which said structured glyceride has an equivalent carbon number of about 32 to about 42.

**48**. A composition according to claim **24** in which said structured glyceride component contains at least 90% by weight triglycerides.

**49**. A composition according to claim **24** in which said structured glyceride component contains at least 60% by weight triglycerides which have an equivalent carbon number of about 32 to about 42.

**50**. A composition according to claim **24** which said structured glyceride is produced using at least one recombinant oil.

**51**. A method for enhancing an animal's absorption of at least one lipophilic compound, said method comprising administering topically to said animal:

(a) at least one lipophilic compound in conjunction with

(b) a structured glyceride component characterized in that it contains some triglyceride species and at least 40% of the triglyceride species have:

(i) about 33 to 70 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms;

(ii) about 30 to 67 wt. % of long chain acyl moieties having more than 12 carbon atoms; and

(iii) an equivalent carbon number of greater than 30 to less than 48.

52. The method according to claim 51 wherein said structured glyceride component predominantly comprises triglycerides.

53. The method according to claim 52 wherein said triglycerides comprise 45 to 65 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms.

54. The method according to claim 52 wherein said triglycerides comprise 35 to 55 wt. % of long chain acyl moieties having more than 12 carbon atoms.

55. The method according to 52 wherein said triglycerides comprise 45 to 65 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms and 35 to 55 wt. % of long chain acyl moieties having more than 12 carbon atoms.

56. A method according to claim 55 in which said at least 60% by weight of said structured glyceride has an equivalent carbon number of about 32 to about 42.

57. A method according to claim 52 in which said structured glyceride has an equivalent carbon number of about 32 to about 42.

58. The method according to claim 51 wherein said lipophilic compound is selected from the group consisting of oil soluble drugs, nutrients and vitamins.

59. The method according to claim 58 wherein said lipophilic compound is a vitamin selected from the group consisting of vitamin A and vitamin E.

60. A method according to claim 58 in which said oil soluble drug is selected from the group consisting of immunosuppressive agents, protease inhibitors, macrolide antibiotics, anesthetics, estrogens, estradiols, progesterone, testosterone, cortisone, phytoestrogens, dehydroepiandrosterone, and growth hormone.

61. A method according to claim 58 in which said vitamins are selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, carotenoids, and lycopenes.

62. The method according to claim 51 wherein the structured glyceride component is prepared by interesterification of an oil containing predominantly medium chain triglycerides and an oil containing predominantly long chain triglycerides in a ratio from about 1:2 to about 2:1.

63. A method according to claim 62 in which said lipophilic compound is an oil soluble drug is selected from the group consisting of immunosuppressive agents, protease inhibitors, macrolide antibiotics, anesthetics, estrogens, estradiols, progesterone, testosterone, cortisone, phytoestrogens, dehydroepiandrosterone, and growth hormone.

64. A method according to claim 62 in which said lipophilic compound is a vitamin selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, carotenoids, and lycopenes.

65. A method according to claim 62 in which said medium chain triglycerides are obtain from fractionated coconut oil, fractionated palm kernel oil, or a mixture thereof.

66. A method according to claim 62 in which said long chain triglycerides are obtained from an oil selected from the group consisting of borage, black currant seed, corn, coconut, canola, soybean, marine oils, fungal oils, safflower, high oleic safflower, sunflower, high oleic sunflower, olive, evening primrose, cottonseed, rice bran, grapeseed, flaxseed, butterfat, garlic, peanuts, almonds, walnuts, wheat germ, egg, sesame, lard, tallow and mutton.

67. The method of claim 51 wherein said structured glyceride component is prepared by chemical interesterification.

68. The method according to claim 51 wherein at least 40% of the triglyceride species have an equivalent carbon number of about 32 to about 42.

69. A method according to claim 51 in which said medium chain acyl moieties have from 6 to 12 carbon atoms.

70. A method according to claim 51 in which said medium chain acyl moieties have from 8 to 10 carbon atoms.

71. A method according to claim 51 in which said structured glyceride has an equivalent carbon number of about 32 to about 42.

72. A composition suitable for topical administration comprising:

(a) at least one lipophilic compound; and

(b) a structured glyceride component characterized in that it contains some triglyceride species and at least 40% of the triglyceride species have:

(i) about 33 to 70 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms;

(ii) about 30 to 67 wt. % of long chain acyl moieties having more than 12 carbon atoms; and

(iii) an equivalent carbon number of greater than 30 to less than 48.

73. The composition according to claim 72 wherein said structured glyceride component predominantly comprises triglycerides.

74. The composition according to claim 73 wherein said triglycerides comprise 45 to 65 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms.

75. The composition according to claim 73 wherein said triglycerides comprise 35 to 55 wt. % of long chain acyl moieties having more than 12 carbon atoms.

76. The composition according to claim 73 wherein said triglycerides comprise 45 to 65 wt. % of medium chain acyl moieties having 4 to 12 carbon atoms and 35 to 55 wt. % of long chain acyl moieties having more than 12 carbon atoms.

77. A composition according to claim 73 in which said structured glyceride has an equivalent carbon number of about 32 to about 42.

78. The composition according to claim 73 wherein the structured glyceride component is prepared by interesterification of an oil containing predominantly medium chain triglycerides and an oil containing predominantly long chain triglycerides in a ratio from about 1:2 to about 2:1.

79. A composition according to claim 78 in which lipophilic substance is an said oil soluble drug is selected from the group consisting of immunosuppressive agents, protease inhibitors, macrolide antibiotics, anesthetics, estrogens, estradiols, progesterone, testosterone, cortisone, phytoestrogens, dehydroepiandrosterone, and growth hormone.

80. A composition according to claim 78 in which said lipophilic substance is a vitamins selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, carotenoids, and lycopenes.

81. A composition according to claim 78 in which said lipophilic compound is an oil soluble drug is selected from the group consisting of immunosuppressive agents, protease inhibitors, macrolide antibiotics, anesthetics, estrogens, estradiols, progesterone, testosterone, cortisone, phytoestrogens, dehydroepiandrosterone, and growth hormone.

82. A composition according to claim 78 in which said lipophilic compound is a vitamin selected from the group consisting of vitamin A, vitamin E, vitamin D, vitamin K, carotenoids, and lycopenes.

83. A composition according to claim 78 in which said medium chain triglycerides are obtained from fractionated coconut oil.

84. The composition according to claim 72 wherein said structured glyceride is prepared by chemical interesterification.

85. A composition according to claim 84 in which said at least 60% by weight of said structured glyceride has an equivalent carbon number of about 32 to about 42.

86. The composition according to claim 72 wherein said lipophilic compound is selected from the group consisting of oil soluble drugs, nutrients and vitamins.

87. The composition according to claim 86 wherein said lipophilic compound is a vitamin selected from the group consisting of vitamin A and vitamin E.

88. A composition according to claim 86 in which said medium chain triglycerides are obtain from fractionated coconut oil, fractionated palm kernel oil, or a mixture thereof.

89. A composition according to claim 86 in which said long chain triglycerides are obtained from an oil selected from the group consisting of borage, black currant seed, corn, coconut, canola, soybean, marine oils, fish oils, fungal oils, safflower, high oleic safflower, sunflower, high oleic sunflower, olive, evening primrose, cottonseed, rice bran, grapeseed, flaxseed, butterfat, garlic, peanuts, almonds, walnuts, wheat germ, egg, sesame, lard, tallow and mutton.

90. A composition according to claim 86 in which said long chain triglycerides are obtained from fish oil.

91. The composition according to claim 72 wherein at least 40% of the triglyceride species have an equivalent carbon number of about 32 to about 42.

92. A composition according to claim 72 in which said medium chain acyl moieties have from 6 to 12 carbon atoms.

93. A composition according to claim 72 in which said medium chain acyl moieties have from 8 to 10 carbon atoms.

94. A composition according to claim 72 in which said structured glyceride has an equivalent carbon number of about 32 to about 42.

95. A composition according to claim 72 in which said structured glyceride component contains at least 90% by weight triglycerides.

96. A composition according to claim 72 in which said structured glyceride component contains at least 60% by weight triglycerides which have an equivalent carbon number of about 32 to about 42.

97. A composition according to claim 72 in the form of a transdermal patch, lotion or ointment.

98. A composition according to claim 72 which said structured glyceride is produced using at least one recombinant oil.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

# CERTIFICATE OF CORRECTION



PATENT NO. : 6,160,007
DATED : December 12, 2000
INVENTOR(S) : DeMichele et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Claim 17, should read:

-- a method according to claim 12, in which said long chain triglycerides are obtained from an oil selected from the group consisting of borage, black currant seed, corn, coconut, canola, soybean, marine oils, fungal oils, safflower, high oleic safflower, sunflower, high oleic sunflower, olive evening primrose, cottonseed, rice bran, grapeseed,flaxseed, butterfat, garlic, peanuts, almonds, walnuts, wheat germ, egg, sesame, lard, tallow and mutton. --

Claim 78, should read:

-- the composition according to claim 72 wherein the structured glyceride component is prepared by interesterification of an oil containing predominantly medium chain triglycerides and an oil containing predominantly long chain triglycerides in a ratio from about 1:2 to about 2:1.

Signed and Sealed this

Twelfth Day of February, 2002

*Attest:*

*Attesting Officer*

JAMES E. ROGAN
*Director of the United States Patent and Trademark Office*

# EXHIBIT I

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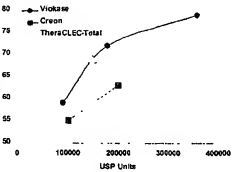
(54) Title: LIPASE-CONTAINING COMPOSITION AND METHODS OF USE THEREOF




(57) Abstract: Disclosed are compositions including crosslinked lipase crystals that are highly resistant to proteolysis, low pH and elevated temperature.

WO 01/62280 A2

# LIPASE-CONTAINING COMPOSITION AND METHODS OF USE THEREOF

## FIELD OF THE INVENTION

This invention relates generally to compositions containing enzymes and more particularly to compositions containing lipase for the treatment of disorders characterized by low lipase secretion.

## BACKGROUND OF THE INVENTION

Metabolic or gastrointestinal diseases often result from the absence of an effective enzyme whose function is necessary at a particular point in a biochemical pathway. For example, improper lipase levels can be traced to a variety of digestive disorders, including fat malabsorption. Fat malabsorption often develops in patients suffering from cystic fibrosis, chronic pancreatitis, and other diseases of the pancreas when pancreatic lipase secretion falls below 5-10% of normal levels. Commonly observed consequences of fat malabsorption include abdominal discomfort, steatorrhea (fatty stools), essential fatty acid (EFA) deficiency, fat-soluble vitamin (*e.g.*, A, D, E, and K) deficiency, and a generalized failure to thrive.

One recognized method for treating diseases or conditions associated with lipase insufficiency is oral replacement therapy. This treatment regimen includes orally administering lipase enzymes to an afflicted individual to increase digestion and a subsequent absorption of nutrients. Commercially available preparations of lipase can fail to completely treat symptoms associated with lipase insufficiency. For example, commercially available porcine lipase can fail to eliminate pancreatic steatorrhea caused by chronic pancreatitis or cystic fibrosis. Factors responsible for difficulties in the treatment of steatorrhea can include destruction of substituted lipase by gastric juice, destruction of substituted lipase by intraluminal proteases, and asynchronous gastric emptying of enzyme supplement and meal nutrients.

Lipases commonly used in replacement therapy are most active at an alkaline pH, and show significant loss of activity when the pH is less than 5. Pancreatic lipase, for example, has been reported to be irreversibly denatured at pH 4 or below. Because of this instability, lipase-based replacement therapies can include repeated administrations of lipases and/or administration of high doses of the enzymes to afflicted individuals. High doses of the enzymes can be associated with undesirable side effects.

## SUMMARY OF THE INVENTION

The invention is based in part on the discovery of compositions which include lipase in a crosslinked crystalline form that is highly resistant to proteolytic and acid degradation. Because the crosslinked crystalline lipase exhibits high stability against proteases and acid, the composition can be administered in low doses to patients suffering from gastrointestinal disorders. In one aspect the invention provides a composition that includes a crosslinked lipase crystal, a protease, and an amylase. The lipase crystal in the composition is crosslinked with a multifunctional crosslinking agent and is preferably stable at pH 1-9. Preferably, the enzyme is active at a pH range from about 2.0 to 9.0. More preferably, the enzyme is active at pH 4-7.

A preferred composition includes a cross-linked enzyme *Burkholderia cepacia ("BC")* crystal, a fungal or plant protease, and a fungal or bacterial amylase. Preferably, the protease is bromelain.

Preferably, the lipase crystal is active following exposure of the lipase crystal for extended periods of time to proteases, acidic conditions, elevated temperatures, or a combination thereof.

Also included in the invention is a method for treating or preventing fat malabsorption in a mammal, *e.g.*, a human, who suffers from, or is at risk for, a condition characterized by low lipase activity. The method includes administering to the subject a composition that includes a crosslinked crystal of a lipase, a protease and an amylase, in an amount sufficient to prevent or inhibit low lipase activity, or to reduce or prevent symptoms associated with low lipase activity.

The highly stable lipases described herein are stable upon administration to a subject. Thus, they can be administered in the absence of enteric-coated microsphere preparations. The lipases described herein can also be administered in lower doses to a subject.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a histogram showing the effect of various doses of cross-linked *Burkholderia cepacia* enzyme complex ("CLEC-BC") on mean coefficient of fat absorption ("CFA").

FIG. 2 is a histogram showing the effect of various doses of CLEC-BC on mean stool fat.

FIG. 3 is a histogram showing the effect of various doses of CLEC-BC on mean coefficient of fat absorption ("CPA").

FIG. 4. is a histogram showing the effect of various doses of a particle containing CLEC-BC, amylase, and a protease on mean coefficient of fat absorption ("CFA").

FIG. 5 is a histogram showing the effect of various doses of a particle containing CLEC-BC, an amylase, and a protease on mean stool fat.

FIG. 6 is a histogram showing the effect of various doses of a composition including CLEC-BC, an amylase and a protease on mean coefficient of protein absorption ("CPA").

FIG. 7 is a graph showing the effect of various therapeutic lipases on mean CPA.

## DETAILED DESCRIPTION OF THE INVENTION

The invention provides compositions including crosslinked lipase crystals that are unexpectedly active following exposure to harsh conditions associated with the upper gastrointestinal tract. These conditions include the acidic environment (*i.e.*, the low pH) of the stomach and high levels of proteases present in the gastrointestinal tract. In preferred embodiments, the compositions are provided in long-lasting compositions that pass through the highly acidic gastric environment of the stomach and allow for delivery of the enzymes in the composition to the intestines of a subject.

The amylase and protease components can be provided in crystalline or amorphous, non-crystalline forms. The latter enzymes degrade carbohydrates and proteins present in the intestinal regions.

Because of the enhanced stability of the crosslinked lipase crystals, the pharmaceutical compositions of the invention have a higher specific activities in the gastrointestinal tract. As a result, they can be administered in lower amounts per dose, and can be administered fewer times over the course of a treatment regimen, at lower doses, and in fewer administrations.

*Compositions containing stabilized crosslinked lipase crystals*

The invention provides a composition that includes a lipase crystal, a protease and an amylase. The lipase crystal is preferably present in the composition as a crosslinked crystal.

In general, any lipase can be used in the composition, as long as it can be provided in a crosslinked crystalline form that resists proteolytic degradation and is stable in low pH. In various embodiments, the lipase is provided as a crosslinked crystal that is stable at a pH less than 7, 6, 5, 4.5, 4, 3.5, 3.0, 2.5, 2.0, 1.5 or less. The lipase can be isolated from a prokaryotic or a eukaryotic cell. Preferably, the lipase is from a non-fungal organism. A preferred source of the lipase is a *Pseudomonas* bacterium. If desired, the lipase can be isolated from a cell which expresses a recombinant form of the lipase.

Lipase crystals are grown by methods known in the art, *e.g.* by the controlled precipitation of protein out of aqueous solution, or aqueous solution containing organic solvents, as described in, for example, U.S. Patent No. 5,618,710. For example, lipase crystals can be produced by combining the lipase protein to be crystallized with an appropriate aqueous solvent or aqueous solvent containing appropriate precipitating agents, such as salts or organic agents. The solvent is combined with the lipase at a temperature determined experimentally to be appropriate for the induction of crystallization and acceptable for the maintenance of protein stability and activity. The solvent can optionally include co-solutes, such as divalent cations, co-factors or chaotropes, as well as buffer species to control pH. The need for and concentrations of co-solutes are determined experimentally to facilitate crystallization. In an industrial scale process, the controlled precipitation leading to crystallization can best be carried out by the simple combination of protein, precipitant, co-solutes, and optionally buffers in a batch process. Alternative laboratory crystallization methods, such as dialysis or vapor diffusion can also be adapted. For example, McPherson (Methods Enzymol. 114:112 (1985)), and Gilliland (J. Crystal Growth 90:51-59 (1988)) include a comprehensive list of suitable conditions in reviews of the crystallization literature. Occasionally, incompatibility between the cross-linking reagent and the crystallization medium might require exchanging the crystals into a more suitable solvent system.

Once crystals are grown in a suitable medium, they can be cross-linked. Cross-linking results in stabilization of the crystal lattice by introducing covalent links between the constituent enzyme molecules in the crystal. This makes possible the transfer of enzyme into an alternate reaction environment that might otherwise be incompatible with the existence of the crystal

lattice, or even with the existence of intact undenatured protein. The cross-linking interactions prevent the constituent enzyme molecules in the crystal from going back into solution, effectively insolubilizing or immobilizing the enzyme molecules into microcrystalline structures.

The macroscopic, immobilized, insolubilized crystals can be readily separated from e.g., feedstock containing product or unreacted substrate by simple procedures known in the art, *e.g*, filtration and/or decantation.

Cross-linking can be achieved by a wide variety of reagents, *e.g.*, glutaraldehyde. Cross-linking with glutaraldehyde forms strong covalent bonds between primarily lysine amino acid residues within and between the enzyme molecules in the crystal lattice that constitute the crystal. The crosslinking agent can be a multifunctional crosslinking reagent. Crosslinking agents are described in, for example, the 1999 edition of the Pierce Chemical Company Catalog.

Examples of suitable crosslinking agents include glutareldehyde, succinaldehyde, octanedialdehyde and glyoxal. Additional multifunctional crosslinking agents include halo-triazines, e.g., cyanuric chloride; halo-pyrimidines, e.g., 2,4,6-trichloro/bromo-pyrimidine; anhydrides or halides of aliphatic or aromatic mono- or di-carboxylic acids, e.g., maleic anhydride, (meth)acryloyl chloride, chloroacetyl chloride; N-methylol compounds, e.g., N-methylol-chloro acetamide; di-isocyanates or di-isothiocyanates, e.g., phenylene-1,4-di-isocyanate and aziridines. Other crosslinking agents include epoxides, such as, for example, di-epoxides, tri-epoxides and tetra-epoxides. Such multifunctional crosslinking agents may also be used, at the same time (in parallel) or in sequence, with reversible crosslinking agents, such as dimethyl 3,3'-dithiobispropionimidate•HCl - (DTBP, Pierce), and dithiobis (succinimidylpropionate) (DSP, Pierce).

Formulations and compositions including crystals according to this invention may be crosslinked for additional stability. This allows for the use of such crystals, crystal formulations and compositions in areas of pH extremes, such as the gastrointestinal tract of humans and animals. For example, lipase crystals, may be crosslinked using one of a variety of crosslinkers, including, but not limited to, Dimethyl 3, 3'-dithiobis-propionimidate•HCl (DTBP), Dithiobis (succinimidylpropionate) (DSP), Bis maleimido- hexane (BMH), Bis[Sulfosuccinimidyl]suberate (BS), 1,5-Difluoro-2,4-dinitrobenzene (DFDNB), Dimethylsuberimidate•2HCl (DMS), Disuccinimidyl glutarate (DSG), Disulfosuccinimidyl tartarate (Sulfo-DST), 1-Ethyl-3-[3-Dimethylaminopropyl] carbodiimide hydrochloride (EDC), Ethylene glycolbis[sulfosuccinimidylsuccinate] (Sulfo-EGS), N-[g-maleimidobutyryloxy]succinimide ester (GMBS), N-hydroxysulfo-succinimidyl-4-

azidobenzoate (Sulfo-HSAB), Sulfosuccinimidyl-6-[a-methyl-a-(2-pyridyldithio)toluamido] hexanoate (Sulfo-LC-SMPT), Bis-[b-(4-azidosalicylamido) ethyl]disulfide (BASED) and glutaraldehyde (GA).

In some embodiments, the lipase crystal is provided as a crystal in a powder form. The powder form can be produced, for example, by lyophilization or spray-drying. Lyophilization, or freeze-drying, allows water to be separated from the composition, producing a crystal which can be stored at non-refrigerated (room) temperatures for extended periods of time, and which can be easily reconstituted in aqueous, organic, or mixed aqueous-organic solvents of choice without the formation of amorphous suspensions and with minimal risk of denaturation. Carpenter, et al., Pharm. Res., 14:969 (1997). Lyophilization may be performed as in U.S. Patent No. 5,618,710, or by any other method known in the art. For example, the protein crystal is first frozen and then placed in a high vacuum where the crystalline water sublimes, leaving a protein crystal behind which contains only the tightly bound water molecules.

Because the cross-linked lipases described herein are stable against proteases, the preparations can be formulated in water and provided as aqueous slurry formulations, which is a preferred mode of administering lipases, especially to a pediatric subject.

The catalytic activity of crystallized lipase can be measured using any method known in the art. For example, lipase activity can be determined spectrophotometrically as described in Example 6 of U.S. Patent No. 5,618,710. Lipase activity can be determined by monitoring hydrolysis of the substrate p-nitrophenyl acetate. Substrate cleavage is monitored by increasing absorbance at 400 nm, with an initial substrate concentration of 0.005% and starting enzyme concentration of $1.5 \times 10^{-8}$ M. Lipase enzyme is added to a 5 ml reaction volume containing substrate in 0.2 M Tris pH 7.0 at room temperature. Crystalline lipase is removed from the reaction mixture by centrifugation prior to measuring absorbance.

Alternatively, lipase activity can be measured *in vitro* by hydrolysis of olive oil as describe in Examples 2-4 of U.S. Patent No. 5,614,189.

Lipase activity can also be measured *in vivo*. For example, a small volume (about 3 ml) of olive oil or corn oil can be labeled with $^{99}$Tc-(V) thiocyanate, and crystalline lipase can be labeled with $^{111}$In. The labeled fat is mixed with an animal food on to which is sprinkled the labeled crystalline lipase. Scintigraphic images of the proximal and distal stomach and small intestine are obtained until <5% of the activity remains in the stomach. Emptying curves for each of the isotopes (*e.g.*, percent retention in the stomach over time) and amounts of isotopes

entering the proximal, middle, and distal small bowel from the respective regions of interest are determined.

Preferably, the composition includes a crosslinked crystalline lipase that has a high specific activity. A high specific activity lipase activity is typically one that shows a specific activity to triolein (olive oil) at greater than 500, 1000, 4000, 5000, 6000, 7000, 8000, or 9000 or more units/mg protein.

A preferred lipase is also stable for an extended period of time in a harsh environment found in gastrointestinal regions, *e.g.*, gastric, duodenal and intestinal regions. For example, the lipase is preferably stable for at least one hour in acidic pH, *e.g.*, an environment in which the pH is less than 7, 6, 5, 4.5, 4, 3.5, 3.0, 2.5, 2.0, 1.5 or less.

Alternatively, or in addition, the crosslinked crystalline lipase crystal in the composition is heat resistant. For example, in various embodiments, the crosslinked crystalline lipase in various embodiments is stable for at least one hour at 30 °C, 35 °C, 37 °C, 40 °C, 42 °C or even 45 °C.

Preferably, the composition is stable in the harsh environment, *e.g.*, the acidic environments or high temperature environments, or both, for at least 1, 2, 3, 4, 5, 6, or 12 or more hours.

By "stable" is meant that the lipase crystal is more active than the soluble form of the lipase for the given condition and time. Thus, a stable lipase crystal retains a higher percentage of its initial activity than the corresponding soluble form of the lipase. In some embodiments, the lipase crystal is more active than the non-cross-linked crystalline form of the lipase. In some embodiments, the lipase crystal retains at least 50% of its activity after exposure to the given conditions and time. In some embodiments, the lipase retains 60%, 65%, 75%, 85%, 90%, or more of its activity.

The composition is preferably also provided with a protease. Any protease known in the art can be use in the composition. Preferred proteases are trypsin, bromelain, papain, fungal proteases, or a combination of these proteases.

The composition is preferably also provided with an amylase or with both a protease and an amylase. The amylase can be from any suitable prokaryotic or eukaryotic host. Preferred amylases include those from *Bacillus* or *Aspergillus* species.

Additionally, either the protease, amylase, or both, may be provided in the crystalline form or in a lyophilized form. While the protease, amylase, or both, can be provided in the lyophilized form, in preferred embodiments these are present in non-crystalline, *i.e.*, amorphous, forms.

If desired, additional components can be present in the composition. These components can include, *e.g.*, an esterase.

*Pharmaceutical compositions containing acid-stable crosslinked lipase crystals, a protease, and an amylase*

Also included in the invention is a pharmaceutical composition which includes an acid stable, proteolytic-resistant lipase, a protease and an amylase. Preferably, the lipase is provided in a crystalline form, *e.g.*, a crosslinked crystalline form.

The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals and, more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered.

Typical excipients, include sugars and biocompatible polymers. Examples of excipients are described in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and the Pharmaceutical Society of Great Britain. Representative excipients include sucrose, trehalose, lacitol, gelatin, hydroxypropyl-β-cyclodextrin, methoxypolyethylene glycol, and polyethylene glycol.

If the composition is to be provided in capsule or tablet form, a diluent may be included. Typical diluents include, *e.g.*, calcium carbonate, dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate, microcrystalline cellulose, powdered cellulose, dextrates, dextrin, dextrose excipient, fructose, kaolin, lactose, mannitol, sorbitol, starch, pregelatinized starch, sucrose, compressible sugar, confectionery sugar. Preferably, the pharmaceutical composition is formulated for oral delivery.

In some embodiments, the lipase, protease, and amylase composition is present in the pharmaceutical composition in association with a polymeric carrier. In one embodiment, a slow release composition containing a cross linked crystal lipase is formed. The formulation of crosslinked lipase crystals, lyophilized amylase, lyophilized protease and a polymeric carrier allows for an acid-resistant controlled release capsule that results in delivery of the enzymes in

effective amounts and at low doses to the intestine, *e.g.*, the distal bowel, following oral ingestion. Furthermore, lipase crystals encapsulated within polymeric carriers to form microspheres can be dried by lyophilization.

A polymeric carrier can include, *e.g.*, polymers used for encapsulation of protein crystals for delivery of proteins, including controlled release biological delivery. Such polymers include biocompatible and biodegradable polymers. Preferably, the polymeric carrier is a biodegradable polymer. Biodegradable polymers are polymers that degrade by hydrolysis or solubilization. Degradation can be heterogeneous, *i.e.*, occurring primarily at the particle surface, or homogenous, *i.e.*, degrading evenly throughout the polymer matrix, or a combination of such processes. The polymeric carrier may be a single polymer type or it may be composed of a mixture of polymer types.

To protect the lipase, protease, and amylase from the harsh environment of the gastrointestinal tract, the composition is preferably encapsulated within a matrix of the polymeric carrier.

Microspheres are produced when protein crystals are encapsulated in at least one polymeric carrier to form microspheres by virtue of encapsulation within the matrix of the polymeric carrier to preserve their native and biologically active tertiary structure. The crystals can be encapsulated using various biocompatible and/or biodegradable polymers having unique properties which are suitable for delivery to different biological environments or for effecting specific functions. The rate of dissolution and, therefore, delivery of active protein is determined by the particular encapsulation technique, polymer composition, polymer crosslinking, polymer thickness, polymer solubility, protein crystal geometry and degree and, if any, of protein crystal crosslinking. The crystal(s) may be encapsulated using a variety of polymeric carriers having unique properties suitable for delivery to different and specific environments or for effecting specific functions. The rate of dissolution of the compositions and, therefore, delivery of the active protein can be modulated by varying crystal size, polymer composition, polymer crosslinking, crystal crosslinking, polymer thickness, polymer hydrophobicity, polymer crystallinity or polymer solubility.

In some embodiments, the pharmaceutical composition is provided as a controlled release composition. For example, the composition can be one in which at least 25%, 50%, 75%, 80%, 85%, 90%, or even 95% or more of the composition remains encapsulated within the matrix following exposure of the polymeric carrier to an acidic environment for an extended period of time, *e.g*, an acidic environment having a pH less than 7, 6, 5, 4.5, 4, 3.5, 3.0, 2.5, 2.0, 1.5, or

less for at least one hour. In some embodiments, the composition is retained in the acidic conditions for 2, 3, 4, 6, 10, 12, or 24 or more hours.

In various embodiments, the pharmaceutical composition is administered to a subject prior to, simultaneous with, or following ingestion of food by the subject. The subject to which the composition is administered preprandially, prandially, or postprandially can be, *e.g.*, a human, dog, cat, mouse, rat, horse, cow, or other mammal.

*Therapeutic uses for compositions containing stabilized crosslinked lipase crystals*

Also included in the invention are methods for treating or preventing gastrointestinal disorders in a mammal. According to this method, a therapeutically effective to amount of a crosslinked crystalline lipase, protease, amylase composition is administered to a subject in need of treatment. The subject to can be *e.g.*, a human, dog, cat, mouse, rat, horse, cow, or other mammal. Preferably, the composition is administered orally, *e.g.*, at mealtime. For example, the composition can be administered just before, just after, or while eating.

The compositions of the invention can be used to treat or prevent, for example, pancreatitis, pancreatic insufficiency, fat malabsorption, low lipase secretion, and gastrointestinal complications associated with cystic fibrosis. The methods of this invention can be also be used to treat any condition characterized by inadequate amounts of or ineffective lipase. Such conditions include steatorrhea, essential fatty acid deficiency, failure to thrive, and fat-soluble vitamin deficiency.

The effectiveness of the method of treatment can be assessed by measuring and comparing the coefficient of fat absorption (CFA) in healthy individuals with that of the subject being treated according to the methods of this invention. For example, a healthy mammal has a CFA greater than 90%. Subjects suffering from a gastrointestinal disorder characterized by pancreatic deficiency, pancreatitis, fat malabsorption or low lipase secretion, will typically have a CFA of less than 60%. Preferably, the methods of this invention are employed to increase the CFA of a subject in need of treatment to at least 60%. More preferably, the CFA is increased to greater than 80%. Most preferably, the CFA is increased to greater than 85%.

An alternative means for measuring the efficacy of treatment of a subject according to the methods of this invention is by performing a 72 hour stool test. For example, effective treatment according to the invention decreases stool fat content in an adult human subject to less than 7 grams a day.

The invention will be further illustrated in the following non-limiting examples.

**Example 1. US Pharmacopeia Assay for lipase activity**

The activity of *Burkholderia cepacia* lipase was determined by titrating the released fatty acids from olive oil against sodium hydroxide as described by U.S. Pharmacopeia (Assay for lipase activity in Pancreatin, USP 24, 2000, 1254-1255). The lipase activity in USP units was calculated by comparison to the activity of the standard, using the lipase activity stated on the label of USP Pancreatin Lipase RS. One USP unit of lipase activity is the amount of enzyme that liberates 1.0μ Eq of acid per minute at pH 9.0 and 37 °C under the conditions of the Assay for lipase activity.

**Example 2. Olive oil-based assay for lipase activity**

Lipase activity was measured using an olive oil assay. Lipase supernatant sample were assessed for activity against olive oil in pH 7.7 buffer. The assay was carried out titrimetrically using slight modifications to the procedure described in Pharmaceutical Enzymes - Properties and Assay Methods, Ruyssen and Lauwers, (Eds.), Scientific Publishing Company, Ghent, Belgium (1978).

Solutions used in the assay included the following:

1. Olive oil emulsion: 16.5 gm of gum arabic (Sigma) was dissolved in 180 ml of water and 20 ml of olive oil (Sigma) and emulsified using a Quick Prep mixer for 3 minutes.
2. Titrant : 0.05 M NaOH;
3. Solution A: 3.0 M NaCl;
4. Solution B: 75 mM $CaCl_2.2H_2O$;
5. Mix: 40 ml of Solution A was combined with 20 ml of Solution B and 100 ml of $H_2O$;
6. 0.5% Albumin.

Lipase Substrate Solution

The substrate was prepared by adding 50 ml of olive oil emulsion (solution 1) to 40 ml of Mix (solution 5) and 10 ml of 0.5% albumin (solution 6).

Assay Procedure

The lipase substrate solution (solution 7) was warmed to 37 °C in a water bath. First, 20 ml of substrate was added to a reaction vessel and the pH was adjusted to 7.7 using 0.05 M NaOH (solution 2) and equilibrated to 37 °C with stirring. The reaction was initiated by adding

enzyme. The reaction progress was monitored by titrating the mixture of enzyme and substrate with 0.05 M NaOH to maintain the pH at 7.7.

The specific activity (μmoles/min/mg protein) was equal to the initial rate X 1000 X concentration of the titrant/the amount of enzyme. The zero point was determined by running the reaction without enzyme, i.e., using buffer in the place of enzyme in the reaction mixture. The initial rate was equal to base consumption in ml/ time in min. The blank was a sample without enzyme, i.e., buffer was used instead of enzyme in the reaction mixture.

**Example 3. Assay for protease activity**

The activity of proteases was determined by using casein as a substrate in a procedure as described by U.S. Pharmacopeia (Assay for protease activity in Pancreatin, USP 24, 2000, 1254-1255). The protease activity in USP units was calculated by comparison to the activity of the standard, using the protease activity stated on the label of USP Pancreatin Amylase and Protease RS. One USP unit of protease activity is the amount of enzyme that hydrolyzes casein at an initial rate such that an amount of peptide (that is not precipitated by trichloroacetic acid) is liberated per minute that gives the same absorbance at 280 nm as 15 nmol of tyrosine under the conditions of the assay for protease activity.

**Example 4. Assay for amylase activity**

The activity of amylases was determined using starch as substrate as described by U.S. Pharmacopeia (Assay for amylase activity in Pancreatin, USP 24, 2000, 1254-1255). The amylase activity in USP units was calculated by comparison to the activity of the standard, using the amylase activity stated on the label of USP Pancreatin Amylase and Protease RS. One USP unit of amylase activity is the amount of enzyme that decomposes starch at an initial rate such that 0.16 mEq of glycosidic linkage is hydrolyzed per minute under the conditions of the Assay for amylase activity.

**Example 5. Crystallization of *Burkholderia cepacia* lipase**

The lipase from *Burkholderia cepacia* (lipase PS 3000 - Amano) ("LPS", 150 gm) was dissolved in 1000 mL distilled deionized water and dialyzed against water overnight with three changes. To the protein, tert-butanol was added to a final concentration of 25% and 1M sodium acetate buffer was added to a final concentration of 10 mM, followed by centrifugation to

remove the precipitate that had formed after 1 hour. The crystals of lipase started forming in 15 min. Crystallization was then allowed to proceed for 16 hrs before harvesting. More than 95% yield (based on activity) was obtained by this procedure.

The crystals were rod shaped and fairly uniform in size (approximately 10-15 μm in length) and shape when observed under light microscope, and as measured by a Coulter LS Particle size analyzer.

**Example 6. Crosslinking of *Burkholderia cepacia* lipase crystals**

Crosslinking of lipase crystals was carried out using 2 mM Bis (Sulfosuccinimidyl) suberate BS ("BS") in mother liquor (25% tert-butanol at pH 8.5 in 50mM phosphate buffer). Crosslinking was carried out at 4 °C overnight (16hrs) with tumbling. After 16 hours, the slurry was centrifuged at 3000 rpm and the supernatant was discarded. The crosslinking was terminated by washing off excess reagent with mother liquor in the presence of 10 mM Tris.HCl to inactivate the any unreactive cross-linker. Finally, the cross-linked *Burkholderia cepacia* enzyme complex (CLEC-BC) was washed thoroughly with 10 mM sodium acetate buffer, pH 4.5 and stored at 4 °C.

**Example 7. Measuring crystallinity**

The crystal integrity of the formulations was monitored by inspection under a light microscope and by Coulter counter analysis for particle size measurement. The rod shape of the crystals and their size remained unchanged after crosslinking.

**Example 8. Secondary structure characterization by Fourier transform infrared (FTIR) spectroscopy**

The Fourier transform infrared (FTIR) spectra of soluble, crosslinked, and noncrosslinked lipase crystals were collected on a Nicolet model 550 Magna series spectrometer as described by Dong et al. in Biochemistry 31:9364-70 (1992) and in J. Pharm. Sci.: 84:415-24 (1995). The noncrosslinked lipase crystal slurry and CLEC-BC slurry samples (about 5 to 10 mg/ml each) were placed on a Zinc selenide crystal of ARK ESP. The spectra were collected and processed using Grams 32 from Galactic Software for the determination of relative areas of the individual components of secondary structure using second derivative and curve-fitting program under the amide I region (1600 –1700 $cm^{-1}$). Both noncrosslinked soluble lipase and CLEC-BC gave identical spectra without any major changes in secondary structure.

**Example 9. Activity of cross-linked enzyme *Burkholderia cepacia* ("BC") crystals**

The activity of cross-linked enzyme *Burkholderia cepacia* ("BC") crystals was examined.

CLEC-BC crosslinked with Bis (Sulfosuccinimidyl) suberate (BS) is active. The CLEC-BC crosslinked with BS was approximately ~50% active when compared to noncrosslinked soluble lipase (Table 1). The activities of the soluble lipase and the crosslinked lipase were compared using both the USP method (Example 1) and olive oil-release (Example 2) method.

**Table 1.** Activity of lipase CLEC preparation

| Sample | Specific Activity (Units/mg) |
|---|---|
| 1. Noncrosslinked (Soluble) lipase (USP assay) | 7184 |
| 2. CLEC-BC (olive oil assay) | 3010 |
| 3. CLEC-BC (USP assay) | 1727 |

**Example 10. Activity of CLEC-BC at various pH levels**

The activity of the CLEC-BC was determined at various pH levels using end point titration. The activities were determined at pH 2.0, pH 4.5, pH 5.5, pH 6.5, pH 7.7 and pH 9.0. The samples were titrated using pH STAT for 15 min at the above-mentioned pH levels and then the pH of each sample was immediately raised to pH 7.7, except for the pH 9.0 sample, which was measured as it was. In the cases where the pH was raised to 7.7, the controls were run immediately without incubation for 15 minutes.

CLEC-BC crosslinked with Bis (Sulfosuccinimidyl) suberate-BS was active at various pH levels tested. The CLEC-BC showed activity at various pH ranges. With the exception of pH 2.0, at which only 25% activity was observed, CLEC-BC showed high activity at all pH levels tested.

**Example 11. Stability of CLEC-BC over time**

The stability of CLEC-BC over time was examined. Stability of the CLEC-BC was determined at different pH levels at 37 °C for 5 hours. The CLECs were suspended in pH 2.0

(glycine•HCl buffer), pH 3.0 (glycine•HCl buffer), pH 4.0 (acetate buffer), pH 5.0 (acetate buffer), pH 6.0 (phosphate buffer), pH 7.0 (phosphate buffer), pH 8.0 (phosphate buffer), pH 9.0 (carbonate bicarbonate buffer), and pH 10.0 (carbonate bicarbonate buffer) separately for 5 hrs at 37 °C. Stability of the CLECs was determined by estimating the activities of the CLECs at time zero and at the end of 5 hours. Stability of soluble BC enzyme was also examined at pH 2.0 over a time period of five hours. Activity was measured as a percentage of starting activity.

CLEC-BC was found to be stable at all pH values tested during the time range examined.

**Example 12. Measurement of crystal dissolution in buffer**

The solubility of a BC-CLEC formulation was determined under acidic conditions using 10 mM glycine•HCl buffer, pH 2.0. The CLECs were washed with 10 mM glycine•HCl buffer, pH 2.0, and suspended in the same buffer with tumbling at 37 °C for 5 hr. The crystal dissolution was examined by passing an aliquot through a 0.22 um filter. Protein (Bradford's method) and lipolytic activity (lipase assay using olive oil) were determined in the filtrate (Soluble CLEC) which gave the amount of crystals solubilized. For determining the activity of the crystals (CLEC), the soluble enzyme activity was subtracted from the total activity in the sample (Activity before filtration).

No significant leaching from CLEC-BC was observed at pH 2.0. The solubility of the CLEC formulation was determined under acidic conditions using 10 mM glycine.HCl buffer, pH 2.0. The CLECs were washed with 10 mM glycine•HCl buffer, pH 2.0, and suspended in the same buffer with tumbling at 37°C for 5 hr. Only 0.44% leaching was observed over a period of 5 hours.

**Example 13. *In vitro* assay of bioavailability of CLEC-BC**

Stability against proteolytic degradation was assessed by incubating the CLEC with various proteases, such as pepsin (which is present in the stomach), and trypsin or chymotrypsin (which are present in the duodenum). In addition, protease bromelain was tested because it had been selected to be included in a combination therapy to substitute for protease in the pancreatic extract. Each CLEC was incubated at 37°C under gentle agitation in a solution of either 10 mM glycine•HCl buffer, pH 2.0 for pepsin or 10 mM phosphate buffer for trypsin/chymotrypsin, pH 7.0 or 10 mM acetate buffer, pH 5.5 for bromelain with a CLEC to protease ratio of 10:1 (W/W).

Aliquots were taken at every hour and measured for the residual lipolytic activity using olive oil as substrate.

CLEC-BC showed high stability against pepsin treatment for 5 hours, without any loss of activity or crystal lattice. Under similar conditions, the soluble lipase lost about 58% activity. CLEC-BC showed no loss in activity after 5 hours incubation with trypsin, while soluble enzyme lost about 36% activity in 4 hr under similar conditions. With chymotrypsin, CLEC-BC showed only 28% loss in activity for 5 hours, while soluble lipase lost 82% of activity in 5 hours at pH 7.0. In addition, both CLEC-BC and soluble lipase were stable to proteolytic degradation by bromelain.

**Example 14.** ***In vivo*** **assay of bioavailability of CLEC-BC**

Efficacy and bioavailability studies were performed to demonstrate that administration of particles (5-20 μm diameter) of CLEC-BC will correct steatorrhea in canines with pancreatic insufficiency. Reduction of steatorrhea with CLEC-lipase is related to survival of lipolytic activity. Slowing of gastric emptying, which occurs with high fat meals, enhances the mixing of the lipase with fat that leads to efficient fat digestion and absorption.

For the studies, female mongrel dogs weighing between 18-21 kg were used. Dogs were first anesthetized with an intravenous injection of thiopental sodium and then underwent endotracheal intubation. Anesthesia was maintained by halothane gas. After celiotomy, both the minor and major pancreatic ducts were individually ligated, and all other tissue connections between the duodenum and the head of the pancreas were transected.

During the balance studies, the dogs were fed two meals a day. With the first meal of the study, a carmine red marker was given. After appearance of carmine red in stool, a 72-hour stool collection was started. Fecal consistency (Grade 1: well-formed, Grade 2: mushy or loose, Grade 3: watery) and frequency were recorded and a fecal score was calculated by multiplying fecal consistency and frequency. The 72-hour stool was analyzed for total weight, carbohydrate, fat, and protein. Between studies, the dogs were maintained as described below for at least 3-7 days before beginning another fecal balance study.

Each dog ingested a high fat meal containing 850 Kcal comprised of 21, 43 and 36% of calories, respectively, as carbohydrate, fat and protein. The basic meal was Hill's canned dog food (Hill's Pet Products, Topeka, KS). It contained chicken, meat by-product, rice, ground corn, liver, animal fat, whole egg, turkey, soybean meal and cracked pearled barley. The meal

was supplemented with 46-g promod powder and minerals. A high fat meal (high fat, high protein, and low carbohydrate) was used. In addition, this meal was associated with the best coordination between solid meal emptying and lipase delivery to the duodenum. Suzuki et al., Gastroenterology 112:2048-55 (1997); Cornell, Experiments with mixtures New York:Wiley, (1981); Boivin et al., Gastroenterology 99:1763-1771(1990). To adjust the mineral content so that the mineral requirement per day was nearly equal among the meals, 1.7g Na3(C6H507) and 2.0g KCl was added to the meal. Overall content of mineral was as follows: Ca-2088 mg, Na-1170mg, K-2108mg, Cl-1869mg, P-1699mg, Mg-180mg.

Between studies, dogs were fed canned dog food (Hill's prescription diet, canine i/d, Hill's Pet Products, Topeka, KS). Each can contained 580 Kcal, comprised of 48% carbohydrate, 27% fat and 25% protein as percentage of calories, 15 g of fat as triglyceride, diglyceride, monoglyceride and fatty acid, 1 g of cholesterol and 1 g of cholesterol ester, and 0.5 g of phospholipid. Dogs were fed two cans in the morning and one can in the afternoon. Ten grams of porcine pancreatin powder (Viokase, AH Robins Company, Richmond, VA) were given with the morning meal and 7 g with the afternoon meal. This dose of pancreatic enzymes maintains the body weight of pancreatic insufficient dogs within 10% of preoperative values. Dogs were weighed weekly. Fasting blood glucose levels were measured weekly.

The results are presented in FIGS. 1, 2, and 3. CLEC-BC was administered at doses of 150,000 units ("Thera CLEC-BC" (1)" in FIGS. 1-3); 30,000 units ("Thera CLEC-BC" (2) in FIGS. 1-3), or (7,500 units "Thera CLEC-BC" (3) in FIGS. 1-3). FIG. 1 shows the effect of various doses of CLEC-BC on mean coefficient of fat absorption (CFA). Post-operative mean CFA levels in untreated dogs was reduced to about 60% of pre-operative levels. For all doses tested, addition of CLEC-BC restored percent CFA to about 90% of pre-operative levels.

FIG. 2 shows the effect of various doses of CLEC-BC on mean stool fat. In untreated post-operative dogs, mean stool fat increased from barely detectable levels to 40 grams/24 hours. Addition of CLEC-BC to post-operative dogs decreased mean stool fat to about 10 grams/24 hours for all doses tested.

FIG. 3 shows the effect of various doses of CLEC-BC on mean coefficient of protein absorption (CPA) in four dogs. Mean CPA decreased from about 95% absorption in pre-operative dogs to about 40% in post-operative dogs. Addition of CLEC-BC did not significantly affect mean CPA. CLEC-BC achieved reductions in CFA comparable to those observed using VIOKASE® and CREON®, two agents used to treat pancreatic exocrine insufficiency.

However, CLEC-BC differed from the agents in the amount of dose required to correct steatorrhea in dogs. Similar effects were achieved by using only 6-113 mg CLEC-BC vs. 1-4 g VIOKASE® and 0.5-1.0 g of CREON®. It can be seen from FIG. 3 CLEC-BC did not increase CPA over its postoperative, untreated level.

**Example 15.** ***In vivo*** **assay of bioavailability of a composition including CLEC-BC, bromelain, and amylase**

The bioavailability of a composition including CLEC-BC, bromelain, and amylase in correcting pancreatic azotorrhea was examined. Efficacy was compared to efficacy of addition of the lipases VIOKASE® and CREON®.

The coefficient of protein absorption (CPA) following addition of the agents was measured by 72-hr fecal balance studies immediately before and after the operation resulting in pancreatic insufficiency, and 3 weeks after the operation with the doses of the following products: VIOKASE® 8, 4 and 2 tablets (240,000 120,000 and 60,000 USP protease units); CREON-20® 2 and 1 capsules (150,000 and 75,000 USP of protease); CLEC-Total consisting of CLEC-BC (150,000 USP; 113.5 mg), bromelain (150,000 USP; 214mg) and *Bacillus* amylase (150,000 USP; 71.9 mg). The enzyme activity of these preparations is shown in Table 2.

During these studies, dogs ingested a diet comprised of 43% fat, 36% protein and 21% carbohydrate as a percentage of calories (1700 kCal/d).

The coefficient of fat absorption was > 88 % with all treatments (FIG. 4). The coefficient of protein absorption (CPA) was directly correlated ($r = 0.86$) with the amount of protease in the preparations. The CPA increased from 59% with the lowest protease dose to 79% with the highest protease dose. The CPA with bromelain (69%; 150,000 USP Protease) was similar to the CPA of 2 capsules of CREON® (63%; 150,000 USP units) and to the CPA of 4 tablets of VIOKASE® (72%; 120,000 USP protease units) (FIGS. 6 and 7). It was noted that the actual proteolytic activities of the VIOKASE® and CREON® were at least 35% higher than their stated activities. Indeed, the actual protease activity of VIOKASE® was 46,248 USP units per tablet *vs* the 30,000 USP units per tablet claimed for VIOKASE® , and 100,519 USP units per capsule *vs* the 75,000 USP units per capsule claimed for CREON® .

**Table 2. Activities of lipase, protease and amylase in various therapeutic preparations.**

| | Number of Tablets/ Capsules | Weight (mgs) | Lipase (USP units) | CFA % | Protease (USP units) | CPA % | Amylase (USP units) |
|---|---|---|---|---|---|---|---|
| Viokase | 8 | 3440 | 64,000 | 88 | 369,984 | 79 | 404,352 |
| | 4 | 1720 | 32,000 | 88 | 184,992 | 72 | 202,176 |
| | 2 | 860 | 16,000 | 89 | 92,496 | 59 | 101,088 |
| Creon® | 2 | 944 | 40,000 | 89 | 201,038 | 63 | 219,420 |
| | 1 | 472 | 20,000 | 90 | 100,519 | 55 | 109,710 |

These results demonstrate that a composition containing CLEC-BC, bromelain, and amylase is at least as effective on a per unit basis as porcine proteases in reducing protein malabsorption (FIG. 6 and 7). In addition, the results show that a small amount (114 mg) of CLEC-BC in CLEC-Total corrects steatorrhea in dogs at least as well as 8 tablets of VIOKASE® (~ 4 gm) or 2 capsules of CRESON (~1 gm) (FIG. 4).

Other embodiments are within the claims.

WHAT IS CLAIMED IS:

1. A composition comprising
a non-fungal lipase crystal crosslinked with a multifunctional crosslinking agent;
a protease; and
an amylase,
wherein the lipase crystal is active at a pH range from about 2.0 to 9.0.

2. The composition of claim 1, wherein the lipase crystal is active at a pH range from about 1.0 to 6.0.

3. The composition of claim 1, wherein the lipase crystal is active at a pH range from about 1.5 to 3.0.

4. The composition of claim 1, wherein the lipase crystal is active following exposure for at least one hour to an environment having pH 1.0 to 4.0.

5. The composition of claim 1, wherein the lipase crystal is active following exposure for at least two hours to an environment having pH 1.0 to 4.0.

6. The composition of claim 1, wherein the lipase crystal is active following exposure for at least five hours to an environment having pH 1.0 to 4.0.

7. The composition of claim 1, wherein the multifunctional crosslinking agent is Bis (Sulfosuccinimidyl) suberate.

8. The composition of claim 1, wherein the lipase crystal is derived from a bacterial lipase.

9. The composition of claim 8, wherein the bacterial lipase is a *Pseudomonas* lipase.

10. The composition of claim 1, wherein the composition is provided in a powder form.

11. The composition of claim 1, wherein the composition is provided as an aqueous slurry.

12. The composition of claim 1, wherein said protease is provided as a crystal.

13. The composition of claim 12, wherein said protease crystal is provided as a cross-linked enzyme crystal.

14. The composition of claim 1, wherein said amylase is provided as a crystal.

15. The composition of claim 14, wherein said amylase is provided as a cross-linked enzyme crystal.

16. The composition of claim 12, wherein said amylase is provided as a cross-linked enzyme crystal.

17. The composition of claim 1, wherein said protease is provided in an amorphous form.

18. The composition of claim 1, wherein said amylase is provided in an amorphous form.

19. The composition of claim 17, wherein said amylase is provided in an amorphous form.

20. The composition of claim 1, wherein the amylase is selected from the group consisting of a *Bacillus* amylase and an *Aspergillus* amylase.

21. The composition of claim 1, wherein said protease is selected from the group consisting of plant and fungal proteases.

22. The composition of claim 1, wherein said protease is selected from the group consisting of bromelain, papain, and ficin.

23. The composition of claim 1, further comprising a pharmaceutically acceptable carrier.

24. The composition of claim 23, wherein the composition is present in a formulation suitable for oral delivery to a subject.

25. The composition of claim 23, wherein the carrier is selected from the group consisting of a diluent, excipient, and adjuvant.

26. The composition of claim 23, wherein the carrier is a polymeric carrier.

27. The composition of claim 24, wherein the polymeric carrier is a biodegradable polymer.

28. The composition of claim 24, wherein the composition is encapsulated within a matrix of the polymeric carrier.

29. The composition of claim 28, wherein at least 50% of the composition remains encapsulated within the matrix following exposure of the polymeric carrier to an environment having pH 1.0 to 3.0 for at least one hour.

30. The composition of claim 24, wherein the composition is administered preprandially, prandially, or postprandially.

31. A composition comprising
a *Burkholderia cepacia* lipase crystal,
bromelain; and
an *Aspergillus* amylase,
wherein the lipase crystal is active at a pH range from about 2.0 to 9.0.

32. The composition of claim 31, wherein said lipase crystal is crosslinked.

33. A method for treating or preventing a gastrointestinal disorder in a mammal, the method comprising administering to a mammal in need thereof a therapeutically effective amount of the composition of claim 1.

34. The method of claim 33, wherein the composition is administered orally.

35. The method of claim 33, wherein the mammal is a human.

36. The method of claim 33, wherein the gastrointestinal disorder is selected from the group consisting of pancreatitis and pancreatic insufficiency.

37. The method of claim 33, wherein the subject suffers from or is at risk for cystic fibrosis.

38. A method for treating or preventing fat malabsorption in a mammal suffering from or at risk for a condition characterized by low lipase secretion, the method comprising to the mammal the composition of claim 1.

39. The method of claim 38, wherein the composition is administered orally to the mammal.

40. The method of claim 38, wherein the mammal is a human.

41. The method of claim 38, wherein the composition is administered preprandially to the subject.

42. The method of claim 38, wherein the composition is administered prandially to the subject.

43. The method of claim 38, wherein the composition is administered postprandially to the subject.

44. The method of claim 38, wherein the composition is administered to the mammal in an amount sufficient to increase the coefficient of fat absorption in the mammal to greater than 60%.

45. The method of claim 38, wherein the composition is administered to the mammal in an amount sufficient to increase the coefficient of fat absorption in the mammal to greater than 80%.

46. The method of claim 38, wherein the composition is administered to the mammal in an amount sufficient to increase the coefficient of protein absorption in the mammal to greater than 60%.

47. A method for treating or preventing fat malabsorption in a mammal suffering from or at risk for a condition characterized by low lipase secretion, the method comprising to the mammal the composition of claim 31.

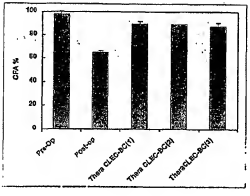



FIG. 1

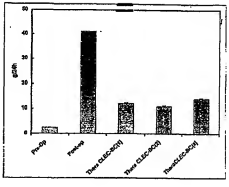



FIG. 2

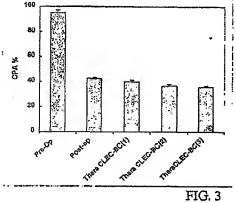



FIG. 3

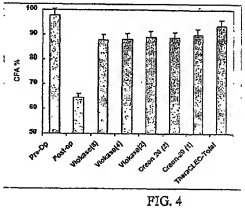



FIG. 4

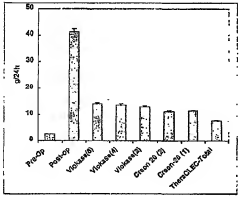



FIG. 5

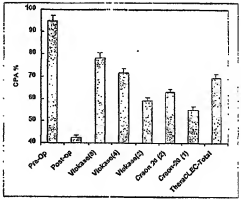



FIG. 6

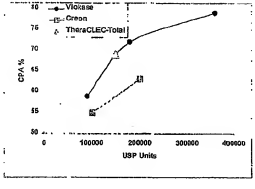



FIG. 7

# EXHIBIT J

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(54) Title: NUTRITIONAL COMPOSITION

(57) Abstract: A composition for a nutritional supplement for convalescing patients recovering from illness or surgery, those with limited appetite such as the elderly, children or anorexic patients, or those who have impaired ability to digest other sources of protein such as persons having chronic gastritis who have a reduced gastric pepsin digestion. The supplement comprises: (i) a protein source which provides at least about 8% total calories of the composition and which includes at least about 50% by weight whey protein; (ii) a lipid source having an omega 3:6 fatty acid ratio of about 5:1 to about 10:1 and which provides at least about 18% total calories of the composition; (iii) a carbohydrate source; and (iv) a balanced macronutrient profile comprising at least vitamin E and vitamin C. The supplement has reduced capacity to induce satiety. Also disclosed are a method of production of the composition; use of the composition in the manufacture of a functional food or medicament; and a method of treatment which comprises administering an effective amount of the composition.

WO 02/15719 A2

# SPECIFICATION

## TITLE

## "NUTRITIONAL COMPOSITION "

This application claims the benefit of U.S. Provisional Application No. 60/227,117 filed on August 22, 2000.

## BACKGROUND OF THE INVENTION

The present invention relates to a composition for a nutritional supplement; a method of production of the composition; use of the composition in the manufacture of a functional food or medicament for the nutrition, prevention or treatment of convalescing patients recovering from illness or surgery, those with limited appetite such as the elderly, or anorexic patients, or those who have impaired ability to digest other sources of protein such as persons having chronic gastritis who have a reduced gastric pepsin digestion; and a method of providing nutrition or treatment which comprises administering an effective amount of the composition.

Many people do not take in sufficient nutrients for a nutritionally complete diet. In order to assist these people, nutritional supplements have been developed. Nutritional supplements are usually not intended to provide all the nutrients necessary for a nutritionally complete diet; instead they are generally intended to supplement the diet such that it becomes more nutritionally complete. However, in some instances they may provide complete nutrition.

There are many targets for nutritional supplements; for example sick patients, convalescing patients, anorexic patients and the elderly. For sick and convalescing patients, the spontaneous intake of food is often lower than normal and insufficient to meet nutritional needs. Recovery and restoration of strength may therefore be impaired. A significant proportion of the elderly, on the other hand, tend to eat too little to meet all of their nutritional needs. This is usually due to reduced energy needs following reduction in body weight and diminished physical activity. Anorexic patients by definition suffer a loss of appetite and do not take in sufficient nutrients. In all cases, supplementation to provide missing nutrients can offer advantages.

Various nutritional supplements are available. A family of supplements commonly found in North America is sold under the name ENSURE by Ross Laboratories. The protein source used is predominantly caseinate and soy protein isolates. Another family which is commercially available is sold under the name RESOURCE by Novartis Nutrition Ltd. In this case, the protein source is based on caseinates. Another family which is commercially available is sold under the name NUBASICS by Nestlé Clinical Nutrition. In general, the protein source used in products of this family is caseinate. However, it is found that these products suffer from the problem that they do not necessarily result in a consumer receiving sufficient nutrients; either because an insufficient amount of the product is consumed or insufficient other foods are consumed. This is especially the case with convalescing patients, the elderly and other anorexic patients where loss of appetite leads to insufficient nutrients being consumed.

Nutritional supplements which are based on other protein sources, such as whey protein, are also available or have been described in the literature. In general, the nutritional supplements based upon whey protein are provided in the form of fruit juices; for example as described in European patent application 0486425 and US patent 5,641,531. However, these products suffer from the problem that they generally do not provide a lipid source despite the fact that lipids are essential for adequate nutrition.

Therefore, a need exists for a nutritional supplement which contains protein, lipid and carbohydrate sources. In addition, a need exists for a composition that is capable of providing the special nutritional requirements of those with limited appetite such as the elderly or those who have impaired ability to digest other sources of protein such as persons having chronic gastritis who have a reduced gastric pepsin digestion.

## SUMMARY OF THE INVENTION

Remarkably, a composition has now been found that addresses the problems set out above and enables such persons to retain or regain their strength.

In a first aspect the invention provides a composition for a nutritional supplement which comprises (i) a protein source which provides at least about 8%,

total calories of the composition and which includes at least about 50% by weight of whey protein comprising a fraction of at least partially hydrolyzed whey protein; (ii) a lipid source having an omega 3:6 fatty acid ratio of about 5:1 to about 10:1 and which provides at least about 18% of the total calories of the composition; (iii) a carbohydrate source which provides the remaining calories of the composition; and (iv) a balanced macronutrient profile comprising at least vitamin E and vitamin C.

Within the context of this specification the word "comprises" is taken to mean "includes, among other things". It is not intended to be construed as "consists of only".

In a second aspect the invention provides a method of production of the composition which comprises blending the components in the required amounts.

In a third aspect the invention provides use of a composition according to an embodiment of the invention in the manufacture of a functional food or medicament for the nutrition, prevention or treatment of convalescing patients recovering from illness or surgery, those with limited appetite such as the elderly, or anorexic patients, or those who have impaired ability to digest other sources of protein such as persons having chronic gastritis who have a reduced gastric pepsin digestion.

In a fourth aspect the invention provides a method of nutrition, prevention or treatment of convalescing patients recovering from illness or surgery, those with limited appetite such as the elderly, children or anorexic patients, or those who have impaired ability to digest other sources of protein such as persons having chronic gastritis who have a reduced gastric pepsin digestion which comprises administering an effective amount of a composition according to an embodiment of the invention.

Surprisingly it has now been found that a composition for a nutritional supplement in accordance with the invention, because it contains whey protein, is easier to digest and less prone to induce satiety. Therefore the problem of a patient not consuming a sufficient amount of the supplement may be reduced. Similarly, the problem of a patient not consuming sufficient other foods may be reduced. Further, the composition has a well balanced lipid profile which provides a readily available energy source.

Preferably an embodiment of the composition includes whey protein as the primary source of protein (amino acids). The whey protein can be sweet whey or acid

whey or a combination thereof. Preferably it is in the form of a whey protein partial hydrolysate produced by enzymatic treatment, preferably with trypsin, Alcalase or Novozyme, of whey protein and therefore is less viscous, lighter and provides the advantage that it is more easily digested than known compositions for fortified beverages and nutritional supplements.

It should be appreciated that the present invention is not limited to a whey protein hydrolysate. For example, a non-hydrolyzed whey protein can be used, preferably when the composition is made in a powder form.

The partial hydrolysis of whey protein with one or more of the above enzymes could be carried out at a pH ranging from about 6.6 to 8.8 (preferably about 8.5) and a temperature of about 40 to about 70°C (preferably about 65°C) at an enzyme concentration of about 0.5 to about 2.5 (preferably about 1.0) percent of the protein. Preferably, enzyme treatment is carried out for about 5 to about 120 minutes (preferably 15 minutes) to achieve adequate hydrolysis.

Preferably the whey protein hydrolysate represents a minimum of 50% of the protein content in the formulation. It is preferably the sole protein source but may be combined with intact whey protein or other protein or peptide sources including peptides naturally found in whey or milk such as caseino glycomacropeptide (CGMP). Surprisingly, it has been found that, despite the high proportion of partially hydrolyzed protein, in the composition it is physically stable and has a very acceptable taste due to the process used to prepare the hydrolysate and the selection of a flavoring system to give an acceptable organoleptic profile.

Preferably, at least about 50% by weight of the whey protein is hydrolyzed. Most preferably, at least about 70% by weight of the whey protein is hydrolyzed.

Preferably the protein source provides about 8% to about 20%, more preferably an embodiment for adults comprises a protein source which provides about 15% to about 18% (most preferably about 16%) of total energy of the composition. Preferably an alternative embodiment is suitable for children and it comprises a protein source which provides about 8% to about 14% (most preferably about 12%) of total energy of the composition.

Remarkably, because of the nature of the whey protein and the fact that it is capable of being easily digested, the composition has a beneficial effect in persons

requiring a nutritional supplement such as those recovering from illness or surgery, those with limited appetite such as the elderly or those who have problems digesting other sources of protein such as persons having chronic gastritis who are known to have a reduced gastric pepsin digestion. Remarkably, the composition enables such persons to retain or regain their strength quickly and therefore helps aid recovery of a convalescing patient.

Preferably the lipid source comprises about 40% to about 65% by weight of monounsaturated fatty acids; and about 15% to about 30% by weight of polyunsaturated fatty acids. The saturated fatty acid content is preferably less than about 30% by weight. Up to 20% by weight of medium chain triglycerides may be incorporated into the fat blend to facilitate digestion. The lipid source may contain at least about 30 mg of vitamin E per 100 g of lipid source.

Preferably the lipid source provides about 25% to about 35% of total energy of the composition, more preferably about 30% of total energy of the composition.

Preferably the carbohydrate source comprises sucrose, corn syrup, maltodextrin or a combination thereof. Preferably the carbohydrate source provides about 50% to about 60% of total energy of the composition.

Preferably, an embodiment of the composition has a micronutrient composition having a unique profile rich in nutrients including one or more selected from the group which comprises Vitamin E, Vitamin C, taurine, folic acid and vitamin B-12. Remarkably, the profile aids replenishment of nutrients required in higher quantities during periods of illness or recovery due to oxidative stress or inflammatory conditions and nutrients such as vitamin B-12 that may be poorly absorbed in those suffering from digestive disorders such as chronic gastritis or those who have undergone major intestinal surgery.

Preferably the micronutrients include at least folic acid and/or Vitamin B-12.

Preferably an embodiment of the composition comprises prebiotic fiber. Preferably the fiber is selected from the group which comprises inulin, acacia gum, resistant starch, dextran, xylo-oligosaccharide (XOS), fructooligosaccharide (FOS), galactooligosaccharide or a combination thereof.

Preferably an embodiment of the composition is in a powdered form for dilution with water before use or a ready to use fortified beverage in liquid form; or in the form

of a pudding with a custard or flan like texture suitable for consumption by those with dysphagia or other swallowing problems; or in the form of a bar to provide an interesting selection of different varieties.

Preferably, an embodiment of the composition is formulated for human consumption and/or administration. Preferably, an alternative embodiment is formulated for consumption by a companion animal.

Preferably, an embodiment of the composition according to the present invention comprises the addition of at least one probiotic micro-organism. The probiotic micro-organism provides the advantage of restoring the natural balance of the intestinal flora following antibiotic therapy.

More preferably an embodiment of the composition in powdered form contains a lactic acid bacterium and/or its fermentation metabolites. Preferably the lactic acid bacterium is selected from the group which consists of L. johnsonii, Lb. Paracasei or a combination thereof. This product has the advantage of inhibiting the growth of H.pylori in the stomach which is associated with the development of ulcer particularly in individuals having gastritis. Most preferably the probiotic bacteria comprises a Lb. Paracasei strain deposited under the number NCC 2461.

An advantage of the present invention is that it provides a composition that can be provided in a functional food product and which therefore does not require special administration.

Another advantage of the present invention is that, in the form of a pudding with a thin custard or flan like texture, the product can be consumed by those suffering from dysphagia.

Another advantage of the present invention is that a preferred embodiment is rich in Vitamin E and Vitamin C and as such can be used to replete levels of these nutrients in the blood following depletion related to infection, sepsis or other oxidative stress. Preferably, an embodiment additionally comprises taurine and as such can be used to replete levels of taurine in the blood following depletion related to infection, sepsis or other oxidative stress.

Another advantage of the present invention is that a preferred embodiment is particularly rich in Vitamin B-12 and folic acid which may be poorly absorbed in patients with gastric disease or following surgery to the intestinal tract.

Additional features and advantages of the present invention are described in, and will be apparent from, the description of the presently preferred embodiments which are set out below.

## DETAILED DESCRIPTION OF THE INVENTION

This invention provides a nutritional supplement which is particularly suitable for providing supplemental nutrition to an elderly patient or other anorexic patient while inducing a reduced level of satiety. Due to its components the supplement is more rapidly digested and therefore the patient is more likely to consume a therapeutically effective amount of the supplement or other food to provide for adequate nutrition.

The protein source includes at least about 50% by weight of whey protein that preferably has been at least partially hydrolyzed. The whey protein used to produce the hydrolysate may be a commercially available whey protein source; either based upon sweet whey or acid whey or a combination thereof. Preferably the whey protein is a whey protein source containing more than 80% by weight of whey protein. A suitable whey protein concentrate is LACPRODAN 9087 and suitable whey protein isolate sources include ALACEN 895 (New Zealand Milk Products Inc), BiPRO (Le Sueur Isolates of Le Sueur, Minnesota), PROVON-190 (Avonmore Ingredients Inc of Monroe Wisconsin) and LACPRODAN 9212 (Royal Proteins, Inc of Rosemont Illinois).

The protein source may, if desired, include amounts of other suitable types of protein. For example, the protein source may further include minor amounts of casein protein, soy protein, rice protein, pea protein, carob protein, oat protein, milk protein, caseino-glyco-macropeptide or mixtures of these proteins. Further, if desired, the protein source may further include minor amounts of free amino acids. The other suitable types of protein preferably comprise less than about 50-% by weight of the protein source; more preferably less than about 30% by weight.

The protein source preferably provides about 8% to about 20% of the energy of the nutritional supplement. For example, the protein source may provide about 15% to about 18% of the energy of the nutritional supplement in an embodiment suitable for

an adult or about 8% to about 14% of the energy of the supplement in an embodiment suitable for pediatric use.

The nutritional composition includes a lipid source. Preferably the lipid source provides about 18% to about 40% of the energy of the nutritional supplement; more preferably 25% to about 35% of the energy of the nutritional supplement. For example, the lipid source may provide about 30% of the energy of the nutritional supplement.

The lipid source may include medium chain triglycerides (MCT) up to a level of 20% of the total lipid by weight. The lipid source is rich in monounsaturated fatty acids. In particularly, the lipid source contains at least about 40% by weight of monounsaturated fatty acids. Preferably, the lipid source contains about 45% to about 65% by weight of monounsaturated fatty acids; for example about 55% by weight.

The lipid source may also contain polyunsaturated fatty acids. Preferably the lipid source contains about 15% to about 30% by weight of polyunsaturated fatty acids; for example about 20% by weight of polyunsaturated fatty acids. The lipid profile of the supplement is preferably designed to have a polyunsaturated fatty acid omega-6 (n-6) to omega-3 (n-3) ratio of about 1:1 to about 10:1. Preferably, the n-6 to n-3 fatty acid ratio is about 5:1 to about 9:1; for example about 7:1.

The lipid source has a saturated fatty acid content of less than about 35% by weight; including medium chain triglycerides. More preferably, the lipid source contains less than about 30% by weight of saturated fatty acids.

Suitable lipid sources include high oleic sunflower oil, high oleic safflower oil, sunflower oil, safflower, rapeseed oil, soy oil, olive oil, canola oil, corn oil, peanut oil, rice bran oil, butter fat, hazelnut oil and structured lipids. Fractionated coconut oils are a suitable source of medium chain triglycerides.

The nutritional supplement also includes a carbohydrate source. The carbohydrate source preferably provides about 40% to about 65% of the energy of the nutritional supplement; especially about 50% to about 60% of the energy of the nutritional supplement. For example, the carbohydrate source may provide about 54% of the energy of the supplement. Several carbohydrates may be used including maltodextrin, corn syrup, corn starch, modified starch, or sucrose, or fructose, or mixtures thereof. If desired, the supplement may be free from lactose.

The nutritional supplement preferably includes a complete vitamin and mineral profile. For example, sufficient vitamins and minerals may be provided to supply about 50% to about 500% of the recommended daily allowance of the vitamins and minerals per 1000 calories of the nutritional supplement. The nutritional supplement preferably is rich in vitamin E. For example, the nutritional supplement may contain between 80 International Units and 120 International Units of Vitamin E per 1000 kcal. More preferably, the nutritional supplement contains about 30 International Units of Vitamin E per 250 ml serving of the supplement. Furthermore the nutritional supplement is also rich in Vitamin C providing between about 150 and about 250 mg per 1000 kcal or preferably about 60 mg per serving. The supplement also preferably contains 200 g of folic acid and 3 g of Vitamin B-12 per serving. Alternative embodiments of the supplement for pediatric use have a modified vitamin and mineral profile specifically tailored to the special needs of this age group.

The nutritional supplement further includes a source of a soluble, prebiotic fiber. A prebiotic fiber is a fiber which beneficially affects the host by selectively stimulating growth and/or activity of bacteria in the colon which have the potential to improve host health. Suitable soluble, prebiotic fibers include fructooligosaccharides (FOS) and inulin. Suitable inulin extracts may be obtained from Orafti SA of Tirlemont 3300, Belgium under the trade mark "Raftiline". Similarly, suitable fructooligosaccharides may be obtained from Orafti SA of Tirlemont 3300, Belgium under the trade mark "Raftilose".

Preferably, both FOS and inulin are provided in a ratio of about 60: about 40 to about 80: about 20, most preferably about 70: about 30. Other possible fibers include gums such as guar gum, xanthan gum, xylo-oligosaccharides, gum arabic, pectin, acacia gum, resistant starch, dextrans or mixtures of these. The fiber selected should not induce satiety.

The soluble, prebiotic fibers are reported to promote the growth of bifidobacteria in the gastro-intestinal tract and, in certain circumstances prevent or decrease the growth of pathogens such as Clostridiae. Further, promoting the growth of bifidobacteria is reported to have various other beneficial effects. Also, during fermentation of the fibers in the colon, short chain fatty acids are produced. These fatty acids are a fuel for intestinal cells.

The soluble, prebiotic fibers are preferably present in an amount sufficient to provide about 4 to about 9 g of soluble, fermentable fiber to the patient per day. Therefore the prebiotic fibers may be present in an amount of about 6 g to about 12 g per 1000 kcal. Alternative embodiments comprise blends of prebiotic fibers in an amount of 9 g or less, for example 4g of blend.

If desired, the nutritional supplement may also contain a source of insoluble dietary fiber. Suitable sources of insoluble dietary fibers are hull fibers from legumes and grains; for example pea hull fiber, oat hull fiber, barley hull fiber, and soy hull fiber.

The nutritional supplement preferably has an energy content of about 800 kcal/l to about 2000 kcal/l; for example an energy content of about 1000 kcal/l or about 1500 kcal/l.

The nutritional supplement may be in the form of a soluble powder, a liquid concentrate, a pudding, a bar/snack or a ready-to-use formulation suitable for oral consumption or enteral administration. Ready to drink formulations are particularly preferred. Various flavors, sweeteners, and other additives may also be present. Artificial sweeteners such as acetosulfame and L-aspartyl based sweeteners may be used; for example acesulfame-K or aspartame or a mixture thereof.

The nutritional supplement may be produced, for example, by blending together the protein source, suspended in water, preferably water, which has been subjected to reverse osmosis, and the lipid source. Commercially available liquifiers may be used to form the liquid mixture If used, emulsifiers may be included in the blend. The vitamins and minerals may be added at this point but are usually added later to avoid thermal degradation. Any added lipophilic vitamins, emulsifiers and the like may be dissolved into the lipid source prior to blending. The liquid mixture is then homogenized; for example in two stages at about 7 MPa to about 40 MPa in the first stage and about 2 MPa to about 14 MPa in the second stage Protein hydrolysis is carried out as described earlier. Alternately, the whey protein may be reconstituted in water and hydrolyzed prior to the formation of an emulsion. This is the preferred procedure if a blend of hydrolyzed whey protein and other intact proteins are desired. If this is the practice adopted, then intact protein and lipid are added to the hydrolyzed whey protein following the hydrolysis procedure and the mixture is then homogenized.

Termination of hydrolysis is achieved by denaturing the enzyme preferably by heat or by adjusting the pH or a combination thereof. Inactivation of the enzyme activity is accomplished by using conditions designed to minimize the detrimental effects of heat on the protein stability and product taste and quality. For example, enzyme inactivation may be achieved by heating to a temperature in the range of about 90°C for 5 minutes to about 110°C for about 15 seconds. This may be carried out by steam injection or by heat exchanger; for example a plate heat exchanger.

The liquid mixture may then be cooled gradually to about 20°C to about 30°C; for example by flash cooling and heat exchanger, preferably a plate heat exchanger. The carbohydrate source may be added at this point or later either in a dry form or as a liquid slurry. The mixture may then be further cooled to add any heat sensitive components; such as vitamins and minerals. Water, preferably water which has been subjected to reverse osmosis, may then be mixed in to form a liquid mixture. The pH and solids content of the homogenized mixture is conveniently standardized at this point.

The liquid mixture may then be thermally treated for example using an aseptic process to reduce bacterial loads and sterilize the product. For example, the liquid mixture may be rapidly heated to a temperature in the range of about 110°C for 5 minutes to about 150°C for about 5 seconds. This may be carried out by steam injection or by heat exchanger; for example a plate heat exchanger. The liquid mixture is then homogenized; for example in two stages at about 7 MPa to about 40 MPa in a first stage and about 2 MPa to about 14 MPa in a second stage.

If it is desired to produce a liquid nutritional supplement, the homogenized mixture is filled into suitable containers, such as cans. The filling may either be aseptic or the containers may be retorted. Suitable apparatus for carrying out filling is commercially available.

Without wishing to be bound by theory, whey protein is believed to be rapidly emptied from the stomach and readily hydrolyzed and absorbed in the intestine. This may result in a shorter post-prandial period in which the patient feels satiated and therefore may result in a rapid return of appetite. On the contrary, proteins like casein, which are slowly emptied from the stomach, provoke a steady, prolonged post-prandial period in which the patient may feel satiated. Therefore the nutritional supplement may

be used to provide supplemental nutrition to elderly and sick and convalescing patients who are prone to anorexia and/or protein-energy malnutrition.

It is also found that the amino acid profile is well suited for promoting endogenous production of glutamine. Therefore the nutritional supplement may be used as an indirect source of glutamine for animals or humans. In particular, the nutritional supplement may be used to provide nutrition to stressed patients having a depleted glutamine status; for example for patients who are critically ill, or who are suffering from sepsis, injury, burns, inflammation, or patients recovering from surgery. Further, the nutritional supplement may be used to promote glutamine synthesis in patients suffering from injured or diseased intestines or to maintain the physiological functions of the intestine. Moreover, the nutritional supplement may be used to maintain or raise plasma glutamine levels in humans and animals and improve immune function.

Further, it is found that the whey protein contains high levels of threonine, an important building block of mucins. Therefore the nutritional supplement has the advantage that it may be used to provide supplemental nutrition to patients suffering from, or at risk of, impaired or reduced mucin production, for example, patients undergoing an inflammatory response, suffering from malnutrition, suffering from cystic fibrosis, malignancy, chronic inflammatory bowel diseases, ulcerative colitis and Crohn's disease, undergoing treatment which includes the administration of non-steroidal anti-inflammatory drugs, and the like, and after total parenteral nutrition.

Further, it is found that the whey protein contains high levels of cysteine, an important antioxidant and immediate procursor of glutathione. Therefore the nutritional supplement has the advantage that it can be used to provide supplemental nutrition to patients suffering from glutathione depletion and low antioxidant status. For example, the nutritional supplement may be used as a nutritional support for elderly or patients undergoing or recovering from acute or chronic inflammatory states.

The amount of the nutritional supplement required to be fed to a patient will vary depending upon factors such as the patient's condition, the patient's body weight, the age of the patient, and other sources of nutrition. However the required amount may be readily set by a medical practitioner. The nutritional supplement may be taken in multiple doses, for example 2 to 5 times, to make up the required daily amount or may be taken in a single dose.

By way of example, and not limitation, examples of the invention are now described for further illustration.

Example 1

A ready-to-drink nutritional supplement is prepared. The nutritional supplement includes the following components:

| Component | Wet weight (% by weight of total composition) | Energy (%) |
|---|---|---|
| Protein<br>Whey protein | 4.8 | 16 |
| Carbohydrate<br>Maltodextrin<br>Sucrose | 13 | 54 |
| Lipids<br>High oleic safflower oil<br>Corn oil<br>Canola oil | 2.8 g | 30 |
| Vitamins and Minerals | At least 5% of RDA | |

The lipid mixture is made up of about 25% by weight of saturated fatty acids, about 55% by weight of monounsaturated fatty acids and about 20% by weight of polyunsaturated fatty acids. The n-6:n-3 ratio is about 7:1. The formula contains 30 IU of Vitamin E and 60 mg of Vitamin C per serving.

The energy density of the supplement is 1000 kcal/l.

Example 2

A ready-to-drink nutritional supplement is prepared. The nutritional supplement contains 16% of calories as protein of which 70% of the protein is hydrolyzed whey and the remainder is intact protein The remaining components are described in Example 1.

The energy density of the supplement is 1000 kcal/l.

Example 3

A ready-to-drink nutritional supplement is prepared. The nutritional supplement contains 16% of calories as protein of which 50% to 100% may be from whey or hydrolyzed whey protein.

The energy density of the supplement is 1500 kcal/l.

Example 4

A powdered nutritional supplement is prepared. The nutritional supplement contains 16% of calories as protein of which 50% to 100% may be whey or hydrolyzed whey protein. The supplement may contain a probiotic bacteria, preferably L. johnsonii.

Example 5 - Pediatric RTD

A ready-to-drink nutritional supplement is prepared that is tailored to the needs of growing children. For example the nutritional supplement contains a lower percentage of calories as protein preferably 10-12% of calories as protein and a higher percentage of calories as carbohydrate. The remaining components are as described in example 1.

The energy density of the supplement is 1000 kcal/l.

Example 6 - Pediatric powder

A powdered nutritional supplement is prepared. The nutritional supplement contains 10-12% of calories as protein of which 50% to 100% may be whey or hydrolyzed whey protein. The supplement may contain probiotics preferably B. bifidus and S. thermophilus

Example 7 - Bar and Puddings

Alternate forms of the supplement are prepared such as puddings and snack bars. The pudding and flan forms are suitable for use by dysphagic subjects. All alternate forms are prepared to contain 10%-16% of calories as protein of which 50% to 100% may be whey or hydrolyzed whey.

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

## Claims

1. A composition comprising,
a protein source, which provides at least about 8 % of the total calories of the composition and which includes at least about 50 % by weight, of the protein source, whey protein,
a lipid source having an omega 3 to 6 fatty acid ratio of approximately 5:1 to about 10:1 and which provides at least about 18 % of the total calories of the composition,
a carbohydrate source and a macro-nutrient profile comprising at least vitamin E and C.

2. The composition according to claim 1, wherein the whey protein includes a partially or essentially fully hydrolyzed whey protein.

3. The composition according to claim 1 or 2, wherein the whey protein includes a whey protein hydrolysate that comprises at least 50 % by weight of the protein source.

4. The composition according to any of the preceding claims, wherein at least 50 % by weight of the whey protein is hydrolyzed.

5. The composition according to any of the preceding claims, wherein the composition includes caseino-glycomacropeptide.

6. The composition according to any of the preceding claims, wherein the protein source provides up to about 20 %, preferably about 10 % to about 20 % of the total energy of the composition.

7. The composition according to any of the preceding claims, wherein the lipid source comprises about 40 % to about 65 % by weight of mono-unsaturated fatty acids

8. The composition according to any of the preceding claims, wherein the saturated fatty acid content is less than about 30 % by weight.

9. The composition according to any of the preceding claims, wherein the lipid source provides about 25 % to about 35 % of the total energy of the composition.

10. The composition according to any of the preceding claims, wherein the carbohydrate source comprises sucrose, corn syrup, maltodextrin and/or a combination thereof.

11. The composition according to any of the preceding claims, wherein the carbohydrate source provides about 50 % to about 60 % of the total energy of the composition.

12. The composition according to any of the preceding claims, including at least one micro-nutrient selected from the group consisting of vitamin E, vitamin C, taurine, folic acid and vitamin B-12.

13. The composition according to any of the preceding claims, wherein the composition comprises at least one prebiotic fiber selected from the group consisting of inulin, acacia gum, resistant starch, dextran, xylo-oligosaccharides, fructo-oligosaccharides (FOS) and/or combinations thereof.

14. The composition according to any of the preceding claims, wherein the composition includes at least one probiotic micro-organism.

15. Use of a composition according to any of the preceding claims as a nutritional supplement.

16. Use of a composition according to any of the claims 1 to 14 in pet food.

17. Use of a composition according to any of the claims 1 to 14 for the preparation of an ingestable carrier for providing nutrition to an individual having an impaired ability to digest proteins.

18. Use of a composition according to any of the claims 1 to 14 for the preparation of an ingestable carrier for providing nutrition to an individual not receiving an adequate amount of nutrition.

19. Use of a composition according to any of the claims 1 to 14 for the preparation of an ingestable carrier for supplementing the nutrition received by an individual having an illness.

20. Use of a composition according to any of the claims 1 to 14 for the preparation of an ingestable carrier for providing nutrition to an individual having a reduced ability to digest protein.

21. The use according to claim 18, wherein the individual is anorexic, elderly, recovering from an illness, recovering from surgery, having a chronic gastritis, having reduced gastric pepsin digestion and/or being convalescing.

22. A method for producing a composition according to any of the claims 1 to 14, which comprises the steps of blending a protein source, a lipid source a carbohydrate source and micro-nutrients.

23. The method according to claim 21, including the step of hydrolyzing the protein source with one or more enzymes at a pH ranging from about 6.6 to about 8.8 and at a temperature of about 40 to about 70 °C at an enzyme concentration of about 0.5 to about 2.5 % of the protein.

24. The method according to claim 23, wherein the hydrolysis of the protein is carried out by enzyme treatment for about 5 to about 120 minutes.

25. The method according to any of the claims 22 to 24, including the step of adding to the product at least one probiotic or prebiotic.

# EXHIBIT K

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | |
|---|---|
| Applicant(s): | Perez-Camargo, et al. |
| Appl. No.: | 10/509,951 |
| Conf. No.: | 3093 |
| Filed: | October 4, 2004 |
| Title: | METHOD OF IMPROVING ABSORPTION OF VITAMIN E BY A PET ANIMAL |
| Art Unit: | 1612 |
| Examiner: | Snigdah Maewall |
| Docket No.: | 3714632-509 |

**DECLARATION UNDER 37 C.F.R. § 1.132**

Sir:

I hereby state as follows:

1. My experience and qualifications are as follows:

   I am a qualified Veterinarian and registered Member of the Royal College of Veterinary Surgeons in the UK. Since 1994 I hold a Ph.D. by the Department of Applied Biochemistry and Food Science, Nottingham University, UK. During the last 10 years at Nestlé, I had the opportunity to gain research experience about changes in the energy and nutritional requirements of cats and dogs along their different life stages. In particular, changes in their digestive system physiology and intestinal microflora. I also developed several veterinary prescription diets by implementing new research findings and knowledge about the interaction between nutrients and disease in cats and dogs.

2. I am one of the named inventors of the above-identified patent application and am therefore familiar with the invention disclosed therein.

3. Independent Claims 35, 52 and 61 recite, in part, an edible composition comprising a pancreatic function-promoter comprising an acidifier, a liver function-promoter comprising taurine ranging between about 0.1% and about 1% by weight of the edible composition on a dry matter basis, and an intestinal mucosa function-promoter comprising fish oil ranging between about 0.1% and 20% by weight of the edible composition on a dry matter basis.

4. Lipid digestion and absorption by the digestive system is a complex process. There are three major steps involved in lipid digestibility:

- Pancreatic Lipolysis: Lipases operate at acidic pH converting fat molecules to molecules that are more easily digested, i.e., from tri-glycerides to mono-glycerides and fatty acids.
- Hepatic (liver) Micellar Solubilization: The products of lipolysis are emulsified with bile acids to form micelles. In felines such as domestic cats, bile acids are conjugated with taurine.
- Intestinal (Jejunal) Absorption: Micelles (little fat drops) diffuse into the intestinal cell wall, where the lipid binds to proteins and the triglyceride is reformed. Fats are incorporated into lipoproteins. Bile salts are then re-circulated.

5. All these steps are necessary to achieve normal fat digestibility (fat digestibility is defined as the percentage of fat that the animal retains and is not excreted in feces). Previous published studies aiming at improving fat digestibility focus only on the first step of the process (Lipolysis), and provide lipase enzymes or a combination of lipase, amylase and protease enzymes as a solution.

6. Over a period of several years, the inventors studied the digestibility of numerous diets in a significant number of cats ($n > 1000$). The inventors did batteries of digestibility studies on a continuous basis following Association Of American Feed Control Officials ("AAFCO") protocols. The inventors found that one third of cats over the age of 12 years ("old cats") suffer from low fat digestibility, i.e., fat digestibility of less than 80%. This level of incidence has never been reported. Based upon these findings, the inventors formed a panel of old cats with low fat digestibility to research specifically the phenomena of age related reduction in fat digestibility in old cats. The results showed that the extent of the reduction in fat digestibility in old cats is quite extreme, ranging from 30% to 80% fat digestibility. This extent of low fat digestibility in the old cat population has not been previously reported. Based upon these results, the inventors investigated the causes of the reduction in fat digestibility in these old

cats. A review of known causes of low fat digestibility indicated that none of the previously described pathologies appeared to be the causative factor in low fat digestibility in old cats.

7. The inventors performed postmortem examinations in those old cats that suffered natural deaths. The findings showed that there is not a single well defined pathology for every cat with reduced fat digestibility, but generally it appears that a combined and parallel degeneration of more than one part of the digestive system is involved in fat digestibility. The organs involved are the pancreas, the liver, and the small intestinal mucosa.

8. Evidence shows that rather than a defined pathology, reduced fat digestibility is part of the normal ageing process of cats. In our studies, increasing age has been highly correlated with decreasing fat digestibility. This is not to be confused with Exocrine Pancreatic Insufficiency ("EPI"), a rare and specific pathology that involves a lack of pancreatic enzymes. EPI can be reversed by the supplementation of the diet with pancreatic extract or purified lipases. Our studies showed that the supplementation of enzymes (lipases) on its own in the diet does not solve the problem of low fat digestibility in old cats.

9. Fat digestibility is crucial for the aging cat because our research also found that old cats depend heavily on their ability to maintain body weight and body condition to delay frailty. Body weight loss is a predictor of death in old cats. Fat is the nutrient that provides the higher calorie concentration in the diet. Fat is also a carrier for liposoluble vitamins (like vitamin E) and essential fatty acids (like arachidonic acid), which are essential for the cat.

10. The cat, unlike the rat, mouse, man, pig and dog, is a compulsory carnivore known for its peculiarities in nutritional requirements. This makes extrapolation of studies between cats and these species risky and prone to wrong conclusions. The reduction levels we have found of fat digestibility in old cats have not been described in any other species.

11. With age, in a complex system like the gut, several different organs and functions can "go wrong" simultaneously and in slow progression, leading to a decreased efficiency

manifested in the form of reduced fat digestibility. Specific diagnosis for each specific condition is not easily available, and a single cure to fix them all impossible. Routine veterinary examinations for old pets generally include renal function, dental health, heart condition, body weight, endocrine and hepatic function. Digestibility is definitely not included in routine veterinary evaluations due to the long time it takes to conduct the examination; digestibility assessment requires 10 days. During the 10 days the diet of the pet must be consistent and accounted for, and during the last 5 days the feces need to be collected and analyzed. Further, the symptomatic presentation of several pathologies of the digestive system can be very similar, making the diagnosis difficult. Even when low fat digestibility is diagnosed, the real etiology of the problem cannot be easily determined.

12. To determine if there was an improvement in fat digestibility in old cats fed different diets containing combinations of pancreatic function promoters, liver function promoters, and intestinal mucosa function promoters, a "wet" diet (Diet A), a "dry" diet, (Diet B), a diet containing a pancreatic function promoter (Diet A + citric acid), a liver function promoter (Diet A + taurine), an intestinal mucosa function promoter (Diet A + fish oil in the form of omega 3 oils), and a combination of the promoters (Diet C) were formulated and fed to cats using the procedure given in Example 1 of the above-identified patent application. The citric acid in the diets was in an amount of approximately 0.1% by weight. The taurine in the diets was in an amount of approximately 0.8% by weight. The fish oil in the diets was in an amount of approximately 3% by weight. The results are shown in the attached Figure 1.

13. Referring to Figure 1, the control diets (Diet A and Diet B) showed a fat digestibility of about 61% and 63% respectively. There was no significant difference between fat digestibility of a wet diet and a dry diet. This confirms that the digestibility of wet and dry diets is substantially the same and that diet is not a factor in evaluating digestibility. Diet A + citric acid, Diet A + taurine, and Diet A + fish oil showed an increase in fat digestibility of 6.6%, 6.1% and 5.5% respectively when compared to the control diets. However, surprisingly, the combination of the three promoters showed a much more pronounced affect on fat digestibility. The combination (Diet C) showed an increase in fat digestibility of 17.5%.

14. In old cats with reduced fat digestibility (<80%), the presence of a single pancreatic function promoter (acidifier), a single liver function promoter (taurine), or a single intestinal mucosa function promoter (omega 3 oils) improved the level of fat digestibility (around 5.5 to 6.6%). However, none of these diets increased the level of fat digestibility above 80%, the level considered as normal. When the inventors provided the same old cats with a diet that contains a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils), the improvement in the level of fat digestibility is more dramatic (around 17.5%). See Figure 1. Only with this diet did the old cats reach a level of fat digestibility that was considered normal (above 80%). This is a dramatic effect; not even in young healthy cats can fat digestibility be 100%. Moreover, no digestive system is 100% efficient (every meal produces some fecal content).

15. The results are surprising and unexpected when the percentage of cats that showed an increase in fat digestibility is analyzed as shown in the attached Figure 2. Referring to Figure 2, the percent of cats that had an improved fat digestibility when administered the promoters in combination was 90%, as compared to the 67% to 75% for the promoters alone. This effect is dramatic. About 20% more cats will have increased fat digestibility if administered a combination or promoters than if administered one of the promoters alone. Thus, one critical discovery is that the number of cats that benefit from a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils) is much greater than the number of cats that benefit from a single promoter. Figure 2 shows that 90% of the cats improved their fat digestibility, versus only 75% when fed a diet with a single pancreatic function promoter (acidifier), 67 % with a single liver function promoter (Taurine), or 67% with a single intestinal mucosa function promoter (omega 3 oils).

16. As further shown by Figure 1 of the above-identified specification, there is a direct correlation between fat digestibility and enhancement of the serum Vitamin E level. In other words, a composition that increases fat digestibility also increases the absorption capacity

of Vitamin E by the body of the animal. As a result, the combination of the pancreatic function promoter (acidifier), the liver function promoter (taurine), and the intestinal mucosa function promoter (omega 3 oils) that increased fat digestibility in the cat can also increase the absorption capacity of Vitamin E by the cat.

17. In conclusion, the decrease in fat digestibility in old cats is a complex problem that involves a decrease in pancreatic function, liver function, and/or intestinal mucosal function. In most cases, as is frequent with old age, there is not a clear and consistent malfunction, but a concomitant and interrupted decrease of multiple organ efficiency or malfunction. The inventors made a critical discovery in that the number of cats that benefit from an edible composition including a combination of a pancreatic function promoter (acidifier), a liver function promoter (taurine), and an intestinal mucosa function promoter (omega 3 oils) is much greater than the number of cats that benefit from a single promoter. The beneficial effects of the edible composition lead to an increase in fat digestibility in the cat that also correlates to an increase in the absorption capacity of Vitamin E by the cat.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this patent and any patent issuing therefrom.

Date: April 5, 2010

Name: Gerardo Perez-Camargo

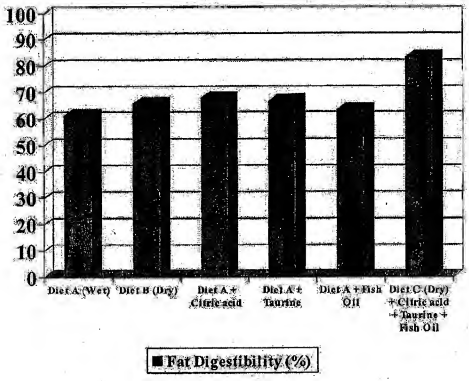
100
90
80
70
60
50
40
30
20
10
0
Diet A (Wet)
Diet B (Dry)
Diet A + Citric acid
Diet A + Taurine
Diet A + Fish Oil
Diet C (Dry) + Citric acid + Taurine + Fish Oil
Fat Digestibility (%)

FIG. 1

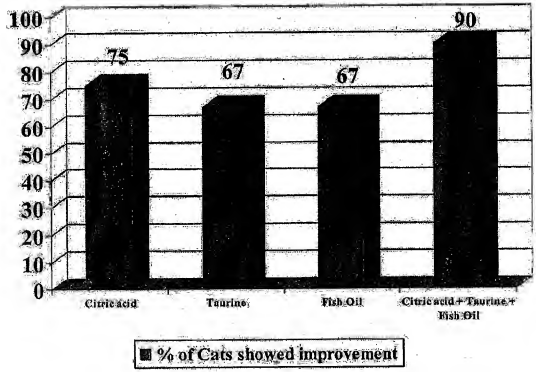
100
90
80
70
60
50
40
30
20
10
0
75
67
67
90
Citric acid
Taurine
Fish Oil
Citric acid + Taurine + Fish Oil
% of Cats showed improvement

FIG. 2